JOURNAL OF AGRICULTURAL RESEARCH

VOL. 41 WASHINGTON, D. C., SEPTEMBER 15, 1930 No. 6

THE NEMIC FAUNA OF THE SLIME FLUX OF THE CAROLINA POPLAR [1]

By G. STEINER

Senior Nematologist, Office of Nematology, Bureau of Plant Industry, United States Department of Agriculture [2]

INTRODUCTION

In Europe the slime flux of various trees is known to harbor certain nemas. The present material, from Shepherdstown, W. Va., is derived from Carolina poplar, *Populus eugenei* Simon-Louis, which is said to suffer badly there from slime flux.

Slime flux is attributed to several agents such as fungi, bacteria, frost, etc. (Ludwig, *4, p. 81, 89, 92;* Neger, *9, p. 111–113;* Tubeuf, *11, p. 142–144.*)[3] Apparently nemas have never yet been considered causal agents, and it seems rather improbable that they should be such. On the other hand, there is the fact that some of these nemic forms have never yet been found elsewhere. However, it is not certain that they are found only in slime flux and have thus to be considered as constant associates. Their modes of distribution are unknown, but it has been suggested that insects that feed on slime flux or are otherwise associated with it may play a rôle in their dispersion. (Ludwig, *4, p. 89,* and Aubertot, *1, p. 334.*) Of course, by such means the whole life association of slime flux might be translocated.

In Europe, *Anguillula dryophila* De Man and *A. ludwigi* De Man were found in the slime flux of the white oak, and *Diplogasteroides spengelii* De Man (*5*), *Mononchus muscorum* (Duj.) Bast., *Dorylaimus silvestris* De Man, *D. oxycephalus* De Man, *D. macrodorus* De Man, and *D. spengelii* De Man in slime flux of the horsechestnut. The former two are closely related to *Anguillula aceti* (Muell.) Muell. found in vinegar, *A. silusiae* De Man in beer (*1*), and *A. nepenthicola* Menzel in cans of *Nepenthes gymnamphora* Nees from Java (*8*). *Diplogasteroides spengelii* was the first known species of its genus; two others were later described by Micoletzky, namely, *D. africanus* Micoletzky from fresh water in South Africa (*6*) and *D. variabilis* Micoletzky from soil soaked with fresh water near Czernowitz (Cernauti), in Bukowina, Rumania (*7*). A fourth, *D. stigmatus*, n. sp., occurs in the present material. It is quite a distinct species belonging to the group with paired ovaries, that is, with *D. variabilis* and *D. africanus; D. spengelii* has only a single ovary. The present slime flux of poplar contains another apparently new species, a Diplogaster that may be named *D. americanus*, n. sp.

[1] Received for publication May 15, 1930; issued October, 1930.

[2] The writer is indebted to Howard H. Somerville, of Shepherdstown, W. Va., for the material used in this study, which reports nemic fauna in slime flux for the first time in the United States.

[3] Reference is made by number (italic) to Literature Cited, p. 433.

Both of these new species were found in larval as well as adult form and undoubtedly pass their whole life cycle in the flux. *Diplogasteroides stigmatus*, however, outnumbered *Diplogaster americanus* as follows:

Diplogasteroides stigmatus.—Larvae, 203; females, 178; males, 127; total number, 508; sexual number, 71.4.

Diplogaster americanus.—Larvae, 15; females, 18; males, 8; total number, 41; sexual number, 44.4.

The nature of the food of the two species found in the present material is not known. It is thought, however, that some other microorganisms (fungi or bacteria) usually present in slime flux may be ingested.

CLASSIFICATION OF THE DIPLOGASTERIDAE

In a previous paper (*10*) it was pointed out that Diplogasteroides is considered to be a good genus, contrary to Baylis and Daubney (*2*). The Diplogaster found in the poplar slime flux belongs to that group of this genus in which a wide anterior portion of the pharynx is followed by a less wide, but not narrow, cylindrical or somewhat conical posterior one; the longitudinally striated anterior portion has a large dorsal or subdorsal onchium. The group contains also *Diplogaster striatus* Bütschli, *D. liratus* A. Schneider, *D. trichuris* Cobb, and others. In the previous paper (*10*) it was pointed out that the present writer fully agrees with Cobb's view (*3*) that the genus Diplogaster, as conceived to-day, includes forms belonging to at least three different groups. An up-to-date revision of this genus might well split it into a number of genera coordinated with Diplogasteroides, Neodiplogaster, Rhabditolaimus, and others. All these are members of the family Diplogasteridae, a family well characterized by the shape of its esophagus and of the male sexual apparatus. The family is interesting because of the variety of ecological conditions under which its members live and also because of the relationship to the Rhabditidae, mononchs, and others. Ecologically, the Diplogasteridae have free-living, saprophytic, saprozoic, plant-parasitic, and animal-parasitic members. It is undoubtedly a group branching out in many directions.

DESCRIPTIONS OF NEW SPECIES

DIPLOGASTEROIDES STIGMATUS, N. SP.

The form of *Diplogasteroides stigmatus* (fig. 1) resembles *D. spengelii* from Europe but may easily be distinguished because the female apparatus is amphidelphic, whereas that of *D. spengelii* is prodelphic. There are a number of other differences, but this one is perhaps the most outstanding. *D. variabilis*, which is also amphidelphic, can at once be distinguished because of the large amphid which is in the latitude of the proximal end of the pharynx. The same is the case with *D. africanus*, which in addition has two ringlike structures at the base of the pharynx.

The head end of *Diplogasteroides stigmatus* is convex-conoid in both sexes, the tail end much elongated and filiform. The cuticle is annulated very finely, but with high magnification this annulation is resolved into transverse series of dots. These, however, are not quite uniform, being larger and more prominent on the lateral body surface, especially in the anal region.

Thus the cuticle of the present species differs from that of the European species, *Diplogasteroides spengelii*, which has longitudinal striae and no special markings on the lateral surface. *D. variabilis* also has longitudinal striae and in addition a lateral wing; and the same striae are found on *D. africanus*, but here the wings are absent. The head end has a single circle of papillae. In *D. spengelii* there is an additional circle of 12 papillalike structures nearer the oral opening;

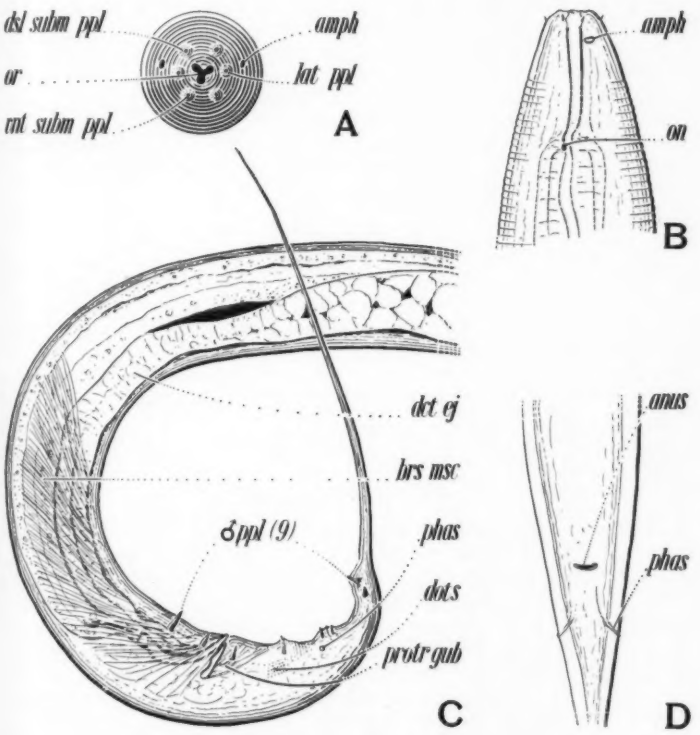

FIGURE 1.—*Diplogasteroides stigmatus*, n. sp.

A.—Front view of head: *amph*, Amphid; *dsl subm ppl*, dorso-submedial papillae; *lat ppl*, lateral papillae; *or*, oral opening; *vnt subm ppl*, ventrosubmedial papilla. × about 1,433
B.—Head end in side view: *amph*, Amphid; *on*, onchium. × about 1,050
C.—Tail end of male in side view: *brs msc*, Bursal muscles; *dct ej*, ductus ejaculatorius; *dots*, dots of the cuticle as they appear along the lateral line; ♂ *ppl (9)*, the nine copulatory papillae of the male; *phas* phasmid; *protr gub*, protractor gubernaculi. × about 533
D.—Ventral view of anal region of female: *anus*, Anus; *phas*, phasmid. × about 533

but De Man says their true nature is uncertain. In the present species no such structures were seen. The amphids of *D. spengelii* are located about opposite the middle of the pharynx; in the present species, however, they are farther forward, almost at the end of the first fourth of the pharynx. The latter is narrower but longer than in the European species. Its walls seem not so strongly cuticularized. A minute toothlike structure was seen at the base of the

cylindrical portion of the pharynx. The esophagus and intestine do not show any variations from the European species, nor do the nerve ring and the excretory pore. The female genital apparatus is amphidelphic. Only one to two eggs are usually seen in each uterus; segmentation has begun when they are deposited. The ovaries are reflexed. The single testis of the male also is reflexed. The spicula are rather slender, arcuate, and cephalated at the proximal ends. The gubernaculum is very different from that of *D. spengelii;* it is longer and more like a spiculum. The arrangement of the muscles of the spicular apparatus may be seen in Figure 1, C. The oblique copulatory muscles are strongly developed and extend about three to four times the length of the spicula in front of the anus. The arrangement of the male copulatory papillae is also different from that of any other Diplogasteroides. A comparison (fig. 1, C) shows best the manner of their arrangement. The phasmid of the male can be located with the help of the female, which also has this structure on its tail. (Fig. 1, D.)

Measurements:

1.9	14	18	20 '46' 17	78	0.9 mm. (0.86–0.94 mm.)
1.5	2.9	3.4	3.8	2.2	
2.2	16	18	48 M	79	0.75 mm. (0.68–0.81 mm.)
2.1	3.2	3.5	4 1	3.2	

Diagnosis.—Diplogasteroides without lateral wings or longitudinal striae but with transverse striae composed of dots which are slightly larger on the lateral fields; without special labial structures, amphids near the end of the first fourth of the pharynx; anterior esophageal bulb well developed; female apparatus amphidelphic; vulva in front of middle; arrangement of male copulatory papillae as shown in Figure 1, C.

DIPLOGASTER AMERICANUS, N. SP.

Diplogaster americanus (figs. 2 and 3) is a large form with a long slender tail. The body tapers only slightly toward the head end, which is broadly convex. The cuticle is annulated at the head end as shown in Figure 2, A; but this annulation soon disappears and 24 longitudinal wings or ridges arise, which can be seen all along the body surface to the beginning of the filiform portion of the tail. Some of these wings fade out as the body begins to taper in the anal region. All the wings are equidistant and appear finely transversely striated, whereas the surface between them seems smooth. This is very strange, because the annulation in front of the wings is complete and continuous. With high magnification this anterior annulation resolves into transverse series of dots, but no such dots are seen on the transversely striated wings. Here, however, the annules are much finer. The dots on the head region are arranged in two series to each annule, one series in the interannular groove and one along the middle of each annule. A single circle of 6 papillae crowns the head, but in addition there are 16 small lips. In the specimens studied they appeared as small rounded elevations surrounding the oral opening and were easily seen in the side as well as in the frontal view. However, it is thought that these lips, when opened and unfolded, will present a different aspect. From the center of each a long, conical, setalike structure runs down the wall of the mouth cavity, giving the latter the appearance of being longitudinally striated. However, it is

thought that all these are labial papillae or setae, normally folded back into the oral cavity, but unfolded and protruded when the nema is feeding. Perhaps they are not sensory organs at all, but of mechanical significance during the intake of food. The pharynx is of irregular shape, the anterior part being wider than the posterior. There is a large slightly subdorsal tooth, curved forward, with strong muscles attached at its base; it is situated at the bottom of the anterior portion of the pharynx. This dorsal tooth, in side view, seems to be opposed by two submedial ventral ones of smaller size and not

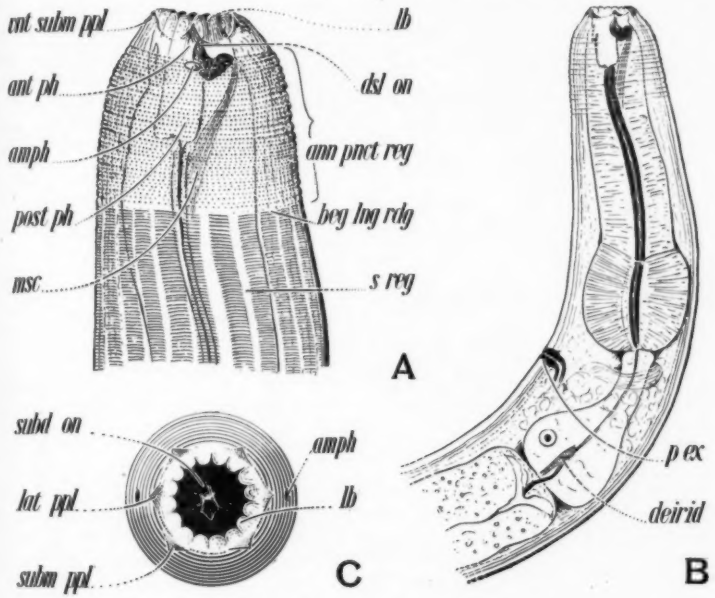

FIGURE 2.—*Diplogaster americanus*, n. sp.

A.—Head end in side view: *amph*, Amphid; *ann punct reg*, annulated and punctate region of cuticle; *ant ph*, anterior portion of pharynx; *beg lng rdg*, beginning of the cross-striated longitudinal ridges on the surface of the cuticle; *dsl on*, dorsal onchium; *lb*, labia (16); *msc*, protruding muscle of onchium; *post ph*, posterior portion of pharynx; *s reg*, smooth surface ribbon between the longitudinal ridges; *vnt subm ppl*, ventrosubmedial papilla. × about 1,433

B.—Anterior end in side view: *deirid*, Deirid located on a longitudinal ridge that is slightly enlarged at this place; *p ex*, porus excretorius. × about 533

C.—Front view of head: *amph*, Amphid; *lat ppl*, lateral papilla; *lb*, labium; *subd on*, subdorsal onchium; *sub ppl*, submedial papilla. × about 1,433

curved; but in a front view nothing could be seen of the latter, and it is therefore concluded that these are only elements of the wall and not real teeth. The anterior portion of the esophagus is extremely strong and thick and followed by a large and muscular median bulb, distinctly set off and with well-developed strong internal walls. The second portion of the esophagus is shorter, well set off from the median bulb, of clear transparent appearance, and with a good-sized posterior bulb containing several nuclei. The intestine consists of large, granulated, polyhedral cells; there are probably only three in a cross section. The excretory pore is well defined and has strongly cutic-

ularized walls; it opens ventrad of the nerve ring. The female sexual apparatus is amphidelphic. The ovaries are reflexed. The male has a single reflexed testis. The male copulatory apparatus consists of two slender spicula, curved and slightly cephalated at the proximal ends. There is a single gubernaculum not quite half as

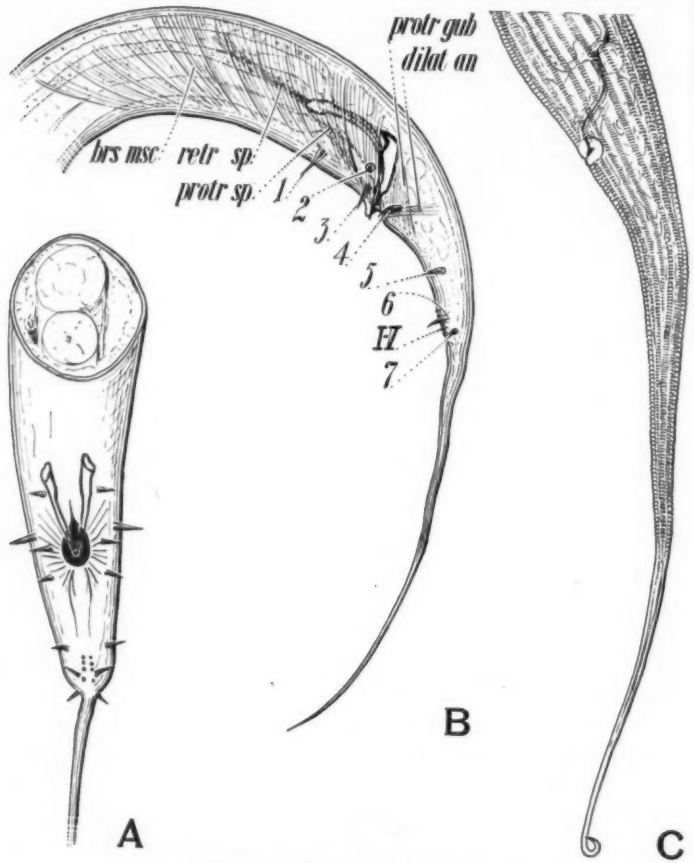

FIGURE 3.—*Diplogaster americanus*, n. sp.

A.—Ventral view of anal region of male, with copulatory apparatus (spicula, gubernaculum, and papillae). × about 533
B.—Tail end of male: *brs msc*, bursal muscles; *dilat an*, dilatator ani; *protr gub*, protractor gubernaculi; *protr sp*, protractor spiculi; *retr sp*, retractor spiculi; *1–7*, large copulatory papillae; *I–V*, small copulatory papillae. × about 533
C.—Tail end of female. × about 533

long as the spicula but much wider and stronger. (Fig. 3, A and B.) The arrangement of the muscles of this copulatory apparatus is best studied on Figure 3, B. The retractor spiculi has its usual position. One protractor spiculi runs from the proximal end of the spicula to the anterior labium of the anus. Another protractor muscle connects the proximal end of the spicula with the inner end of the gubernaculum.

The protractor gubernaculi connects the gubernaculum with the posterior labium of the anus. Numerous bursal muscles are also present, extending at least three to four times the length of the spiculum forward from the anal opening. The male copulatory papillae are very prominent; in fact they have more the shape of strong setae than of papillae. Some are much larger than the others. For their arrangement see Figure 3, A and B. As may be seen, all are ventrosubmedial or ventrolateral.

The present form is undoubtedly closely related to *Diplogaster striatus*, *D. liratus*, and *D. trichuris*. It belongs to the same group as these and may possibly later be brought into one and the same genus or subgenus, when the numerous species of the present genus Diplogaster are revised and reclassified.

Ecologically the members of the present genus Diplogaster include free-living, plant-parasitic, and animal-parasitic forms. "Bridges" exist between the free-living members and the others through saprophytic and saprozoic forms.

Measurements:

3.6	(12)	?	17	24 '46' 18	75	0.85 mm.
3.4	(3.9)	?	4.5	5.7	2.9	
2.2	(12)	?	19	47 'M	74	0.57 mm.
2.4	(5.1)	?	5.4	6.1	4	

Diagnosis.—Diplogaster, the pharynx of which is divided into a wide but short anterior portion at the base of which a large movable slightly subdorsal tooth found, and a less wide but not narrow cylindrical unarmed posterior portion about one and one-half times as long as the anterior. Lips small, 16 in number, with setalike processes, which can be folded into the anterior portion of the pharynx and appear then as longitudinal striae of the pharyngeal wall. Cuticle of the head end annulated, each annule with two transverse striae, one in the interannular groove, the other on the center of the annule; these striae resolve into dots; behind the head end 24 longitudinal, equidistant wings or ridges, which are finely transversely striated, whereas the space between them is smooth; walls of excretory pore heavily cuticularized. Male copulatory papillae as shown.

LITERATURE CITED

(1) Aubertot, M.
1925. Nematodes d'Alsace. Observations sur l'anguillule de la bière (Anguillula silusiae de Man 1914). Bull. Assoc. Philomath. d'Alsace et de Lorraine 6: 333–342, illus.

(2) Baylis, H. A., and Daubney, R.
1926. A synopsis of the families and genera of Nematoda. 277 p. London.

(3) Cobb, N. A.
1924. Neodiplogaster tropica n. g. (?) n. sp. Helminthol. Soc. Wash. Proc., Jour. Parasitol. 11: 105, illus.

(4) Ludwig, F.
1892. Lehrbuch der niederen Kryptogamen, mit besonderer berücksichtigung derjenigen arten, die für den menschen von bedeutung, sind oder im haushalte der natur eine hervorragende rolle spielen. 672 p., illus. Stuttgart.

(5) Man, J. G. de
1912. Helminthologische beiträge. Zool. Jahrb. Sup. 15, Bd. 1: 439–464, illus.

(6) MICOLETZKY, H.
1915. ERGEBNISSE EINER BOTANISCHEN FORSCHUNGSREISE NACH DEUTSCH-ÖSTAFRIKA UND SÜDAFRIKA (KAPLAND, NATAL AND RHODESIEN). SÜSSWASSER NEMATODEN AUS SÜDAFRIKA. Denkschr. K. Akad. Wiss. Wien Math.-Naturw. Klasse 92: [149]–171, illus.

(7) ———
1921. DIE FREILEBENDEN ERD-NEMATODEN. Arch. Naturgesch. Jahrg. 87 (Abt. A): 412–415, illus.

(8) ——— and MENZEL, R.
1928. BEITRÄGE ZUR KENNTNIS DER MICROFAUNA VON NEIDERLÄNDISCH OST-INDIEN. VII. ANGUILLULA NEPENTHICOLA MENZEL AUS KANNEN VON NEPENTHES GYMNAPHORA NEES BEI TJIBODES. Treubia [Batavia] (1928) 10: 285–290, illus.

(9) NEGER, F. W.
1924. DIE KRANKHEITEN UNSERER WALDBÄUME UND DER WICHTIGSTEN GARTENGEHÖLZE; EIN KURZGEFASSTES LEHRBUCH FÜR FORSTLEUTE UND STUDIERENDE DER FORSTWISSENSCHAFT. Aufl. 2, 296 p., illus. Stuttgart.

(10) STEINER, G.
1930. NEODIPLOGASTER PINICOLA N. SP., A NEMA ASSOCIATED WITH THE WHITE-PINE WEEVIL, PISSODES STROBI, IN TERMINAL SHOOTS OF THE WHITE PINE (PINUS STROBUS). Jour. Agr. Research 41: 125–130, illus.

(11) TUBEUF, K. VON
1897. DISEASES OF PLANTS INDUCED BY CRYPTOGAMIC PARASITES; INTRODUCTION TO THE STUDY OF PATHOGENIC FUNGI, SLIME-FUNGI, BACTERIA & ALGAE. Eng. ed. by W. G. Smith. 598 p., illus. London, New York [etc.]

SYNTHETIC NUTRIENT SOLUTIONS FOR CULTURING USTILAGO ZEAE [1]

By EMERY R. RANKER

Formerly *Associate Physiologist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Ustilago zeae (Beckm.) Ung., the organism causing smut of maize, when cultured artificially, usually is grown on a medium that has as a basis a decoction, extract, or infusion of a part of some plant or plant product. Various salts or sugars or both may be added. Potato decoction or carrot decoction or agar is generally used at present as the basis for media in which to culture the fungus. Sartoris [2] recommended a malt extract to which was added peptone, calcium nitrate, and potassium nitrate. Christensen and Stakman,[3] in their work on physiologic specialization and mutation in *U. zeae*, used a potato-dextrose medium.

These various infusions and decoctions are mixtures of soluble plant substances in solution. The specific substances contained in them are unknown for the most part, and of necessity they must be variable, depending upon the variety, conditions of growth, duration of storage, etc., of the material used. It is obvious that such culture media can not be standardized readily and that their exact duplication is uncertain. This is true whether the standardization and duplication are undertaken by different workers in the various laboratories or by the same worker in the same laboratory.

The observations and experiments that have indicated the existence of physiologic specialization in *Ustilago zeae* have preceded the development of cultural media that could be accurately duplicated. It is known that these various physiologic forms differ greatly in appearance when cultured on different media. Most of the results on physiologic forms are based on morphologic characteristics of the fungus in culture. Carrot or potato decoction made from freshly grown material, for example, when compared with a decoction of the same name made from material that has been in storage for several months, may be so different in its physical or chemical properties as to be actually a different medium. A definite conception of physiologic forms of the corn-smut fungus thus grown would be difficult to establish. The results obtained might lead to confusion and the needless multiplication of supposedly different forms. Obviously there is need for some standard culture medium that can be accurately duplicated in any laboratory at any time.

Theoretically, the ideal medium for culturing *Ustilago zeae* should satisfy the following requirements at least: (1) Its constituents

[1] Received for publication Oct. 1, 1929; issued October, 1930.

[2] SARTORIS, G. B. STUDIES IN THE LIFE HISTORY AND PHYSIOLOGY OF CERTAIN SMUTS. Amer. Jour. Bot. 11: 617–647, illus. 1924.

[3] CHRISTENSEN, J. J., and STAKMAN, E. C. PHYSIOLOGIC SPECIALIZATION AND MUTATION IN USTILAGO ZEAE. Phytopathology 16: 979–999, illus. 1926.

should be pure chemicals that would permit accurate duplication of the medium; (2) it should be able to produce, in the same period of time and under the same conditions, an equal or a greater quantity of growth of *U. zeae* than is produced in the generally used potato or carrot decoction; (3) it should have as few constituents as possible consistent with this growth-producing standard; and (4) the constituent chemicals, when added in solution and in culture concentration, should produce little or no precipitate, and the resulting medium should have a hydrogen-ion concentration (preferably after sterilization) favorable to growth without the addition of either acid or alkali to adjust the reaction.

With the above-listed four requirements as a guide, a great many combinations were made. Many were ruled out because of the formation of precipitates that were not dissolved following sterilization of the culture solution. Other combinations were discarded because of unfavorable hydrogen-ion concentration either before or after the growth of the fungus in them. Finally 26 nutrient combinations were chosen for a more detailed study.

MATERIALS AND METHODS

The 26 nutrient solutions listed in Table 1 were tested in detail for growth-producing possibilities in comparison with the control medium, carrot decoction. Most of these solutions were free from precipitates after sterilization, but a few of them produced a negligible precipitate. These nutrient solutions are referred to as "synthetic" in contrast to the decoction mixtures now used generally.

TABLE 1.—*Formulas of 26 synthetic solutions tested for culturing Ustilago zeae*

[Quantities of chemicals expressed as grams in a liter of solution]

Nutrient solution No.	Quantity of indicated chemical																			
	K_2HPO_4	KH_2PO_4	KCl	$NaNO_3$	$MgSO_4.7H_2O$	KNO_3	NaCl	$Ca(NO_3)_2.4H_2O$	K_2SO_4	NH_4NO_3	$CaCl_2$	Na_2SO_4	$Na_2HPO_4.12H_2O$	$CaSO_4.2H_2O$	$CaH_4(PO_4)_2.H_2O$	Ferric phosphate soluble scales	$MnSO_4.2H_2O$	$Mg_3(PO_4)_2.4H_2O$	$FeCl_3$	Dextrose
1	0.25		0.15	0.25	0.2									0.1						10
2	.2		.2	.2	.2									.1						10
3		0.25			.15	0.1	0.15	0.1												10
4	.3				.2	.1	.1	.1												10
5		.15	.2	.2	.15									.1			0.05			10
6	.2		.2	.2											0.1					10
7									0.3	0.1	0.1							0.1		10
8								.2	.2									.2		10
9					.2	.2									.2					10
10		.2			.2	.2	.2							.1						10
11		.2			.2	.2	.2							.1		0.05				10
12		.2			.2	.2	.2							.1		.1				10
13		.2			.2	.2	.2							.1			.05			10
14		.2			.2	.2	.2							.1		.1				10
15		.2			.2	.2	.2							.1		.05	.05			10
16		.2			.2	.2	.2							.1		.1	.1			10
17		.2			.2	.2					.1	0.1								10
18					.2	.25					.15		0.2							10
19					.2	.25					.15		.2			.1				10
20								.2	.2									.2	0.1	10
21					.2	.2									.2				.1	10
22								.2	.2								.1	.2		10
23					.2	.2									.2		.1			10
24								.2	.2								.1	.2	.1	10
25					.2	.2									.2		.1		.1	10
26				.2					.3		.1							.1		10

The chemicals employed in the culture solutions given in Table 1 were measured in cubic centimeters from stock solutions. The stock solutions of the first 13 chemicals, K_2HPO_4 to $Na_2HPO_4.12H_2O$, inclusive, were of such concentration that a unit of 10 c. c. was equivalent to 1 gm. of the salt. The stock solutions of the next 6 chemicals, $CaSO_4.2H_2O$ to $FeCl_3$, inclusive, were of such concentration that a unit of 100 c. c. was equivalent to 1 gm. of the salt. The stock solution of dextrose contained 200 gm. per liter, i. e., a unit of 5 c. c. was equivalent to 1 gm. of sugar.

The same stock solutions were used throughout the experiments, and the same pipettes were used in measuring the required quantities of the respective stock solutions. In making up the various culture solutions about 800 c. c. of distilled water was added to the flask prior to the addition of any of the chemical constituents. This was done to minimize the formation of precipitates. With the exception of solutions Nos. 21 and 25, all those containing both magnesium and iron produced a slight flocculent colloidal precipitate. This precipitate could be resuspended readily on shaking. All other solutions contained a negligible precipitate or none at all after sterilization.

The control solution was a carrot decoction. This was chosen as a standard of measure because of its general use in culturing *Ustilago zeae*. It was made from chopped raw carrots in distilled water. This mixture was autoclaved for 30 minutes at a pressure of 15 pounds, after which the mass was filtered and the filtrate was diluted with distilled water until it was a decoction of 1,000 gm. of carrot roots per liter of solution.

TABLE 2.—*Hydrogen-ion concentrations of the 26 nutrient solutions and of the control, both before and after sterilization*

Nutrient solution No.	pH in relation to sterilization		Nutrient solution No.	pH in relation to sterilization	
	Before	After		Before	After
1	5.9	5.5	14	5.0	4.4
2	5.7	5.4	15	5.3	4.9
3	5.2	4.3	16	5.1	5.0
4	7.2	5.7	17	5.1	4.1
5	5.2	4.0	18	7.0	5.8
6	6.8	5.0	19	6.9	6.2
7	7.4	5.6	20	5.8	4.5
Control	5.0	5.0	21	3.1	3.0
8	7.5	5.5	22	7.0	6.1
9	4.3	4.0	23	4.9	4.1
10	5.0	4.4	24	4.9	4.4
11	4.9	4.8	25	3.1	3.0
12	5.3	4.9	26	7.5	6.0
13	5.0	4.4			

The hydrogen-ion concentrations of the 26 nutrient solutions and of the control (carrot) decoction, before and after sterilization, are given in Table 2. The determinations were made colorimetrically.

After the various nutrient solutions were prepared, 50 c. c. of each was pipetted into each of the desired number of 125 c. c. Erlenmeyer flasks and sterilized in the autoclave for 15 minutes at a pressure of 15 pounds. After sterilization, three to five flasks of each of the nutrient solutions and of the control were inoculated with the smut from a single collection.

Smuts from nine different collections were used. Data on these are given in Table 3. These smuts were grown separately in carrot decoction for six days, during which time they were constantly agitated in a mechanical shaker to prevent growth-lump formation. They were then used as the inoculum, one drop being used to inoculate each of the flasks containing 50 c. c. of the various nutrient solutions. After inoculation the flasks were placed in an incubator maintained at a temperature of 25° C. ±0.5°. Twice each day the flasks were shaken by hand to keep the smut colonies broken up and thus prevent formation of growth lumps.

TABLE 3.—*History and source of the nine cultures of Ustilago zeae used in the experiments on media*

Culture No.	History and source
1ss	Grown from a single sporidium isolated in 1926 from a culture of smut obtained in 1921 from Madison, Wis.
2ss	Grown from a single sporidium isolated in 1926 from a culture of smut obtained in 1922 from Arlington Experiment Farm, Rosslyn, Va.
4ss-2	Grown from a single sporidium isolated in 1926 from a culture of smut obtained in 1923 from Arlington Experiment Farm.
35ss	Grown from a single sporidium isolated in 1926 from a culture of smut obtained in 1925 from Arlington Experiment Farm.
3-2	Isolated from smut-gall tissue collected at Clemson College, S. C., 1927.
34-2	Isolated from smut-gall tissue collected at Mesilla Park, N. Mex., 1927.
50-1	Isolated from smut-gall tissue collected at the Davis County Experiment Farm, Farmington, Utah, 1927.
60-2	Isolated from smut-gall tissue collected at Manhattan, Kans., 1927.
74-2	Isolated from smut-gall tissue collected at University Farm, St. Paul, Minn., 1927.

Each smut was grown in the various nutrient solutions for the periods of time indicated in Table 4. At the end of the period the flasks were removed from the incubator and the quantity of growth that had developed was determined.

Considerable difficulty was experienced in finding a method that would be sufficiently accurate and yet rapid enough to allow making the determinations within a reasonable time. The method of determination by filtration and subsequent weighing of the smut residue and filter paper was found to be impossibly slow. The method of evaporating the liquid from the cultures and then weighing the smut and remaining solids was considered too inaccurate because of the salt and sugar residue that would be weighed with the smut. This error would be greatest with those cultures that had grown least and thus would tend to equalize the results from all solutions.

A combined method, consisting of centrifugation followed by filtration of the supernatant liquid and subsequent weighing of the total residue, was tried. This method was reasonably accurate, but it was slow, and for routine work there were too many operations with the danger of loss of material during transfer. It was found also that this combined method did not produce results that were any more accurate or more uniform than did the method finally selected.

This method consisted of centrifugation at 1,800 revolutions per minute for three minutes, followed by the direct reading of the quantitative results in terms of cubic centimeters of smut thrown down. This centrifuge method was rapid and as accurate as any method tried. Centrifuge tubes were used that were accurately calibrated to 0.10 c. c. and that could be estimated to 0.05 c. c. All the

results are reported as "cubic centimeters of growth obtained by centrifugation."

TABLE 4.—*Average growth of cultures of Ustilago zeae in the control (carrot decoction) and in the 26 synthetic nutrient solutions listed in Table 1*

[Each figure, which is the average of three to five cultures, indicates the cubic centimeters of fungus growth precipitated by centrifugation for three minutes at 1,800 r. p. m. The incubation periods were as follows: No. 3–2, 2 weeks; No. 1ss, 10 days; all others, 3 weeks]

Nutrient solution No.	Average fungus growth produced by cultures of the indicated number								
	2ss	4ss–2	1ss	35ss	3–2	34–2	50–1	60–2	74–2
1	0.5	1.7	0.3	0.3	0.2	0.2	0.2	0.3	0.1
2	.5	1.4	.3	.5	.2	.4	.1	.2	.1
3	.5	1.7	.4	.4	.3	.2	.1	.2	.1
4	.2	1.1	.2	.6	.1	.2	.1	.2	.05
5	.6	1.1	.1	.2	.2	.2	.1	.2	.1
6	.1	.7	.1	.2	.1	.1	.1	.1	.1
7	.7	1.8	.4	.7	.2	.5	.2	.4	.2
8	.6	1.0	.4	.2	.1	.1	.05	.3	.05
9	.5	1.3	.4	.3	.1	.2	.2	.2	.1
10	.5	1.2	.3	.4	.1	.2	.1	.3	.1
11	.6	1.4	.6	.5	.3	.3	.05	.3	.1
12	.4	1.4	.7	.5	.3	.3	.1	.3	.1
13	.4	1.4	.1	.3	.2	.2	.1	.3	.1
14	.5	1.1	.1	.4	.2	.2	.1	.2	.05
15	.3	1.4	.5	.4	.3	.3	.05	.3	.1
16	.3	1.3	.5	.5	.4	.3	.05	.3	.1
17	.4	1.6	.5	.5	.3	.2	.1	.3	.1
18	.4	1.5	.5	.5	.1	.2	.1	.1	.1
19	.1	1.6	.7	.6	.2	.4	.1	.3	.1
20	.4	1.0	.4	.5	.1	.1	.1	.3	.1
21	.5	1.1	.8	.2	.1	.2	.05	.2	.1
22	.2	.6	.1	.3	.1	.2	.1	.3	.05
23	.3	1.2	.2	.4	.2	.2	.1	.3	.05
24	.4	1.1	.2	.4	.2	.1	.05	.1	.05
25	.6	1.0	.8	.3	.2	.3	.05	.2	.1
26	.5	.5	.4	.2	.2	.3	.1	.3	.1
Control	.4	1.3	.5	.4	.....	.2	.1	.4	.1

RESULTS OBTAINED WITH VARIOUS SYNTHETIC SOLUTIONS

The data obtained by growing the various smut cultures in the 26 nutrient solutions tested and in the control (carrot decoction) are given in Table 4. In this table is shown the average growth of each smut in each nutrient solution. There are 242 averages to be compared, 1 being missing from the total of 243. Of these, and for each respective smut, 226 of the average quantities of growth were less than or only equal to those produced in solution No. 7. In only 16 of the 242 averages were the respective average quantities of growth greater in some other nutrient solution than in No. 7. In all 16 cases in which the average growth was less in solution No. 7 than in some other nutrient solution the smut culture was either No. 1ss or No. 3–2. In but 1 case (smut No. 1ss) did the average growth in the control solution (carrot decoction) exceed that in solution No. 7.

An analysis of the data in Table 4 indicates, therefore, that synthetic nutrient solution No. 7 is a superior medium for culturing *Ustilago zeae*. A consideration of Figure 1 confirms this indication. In Figure 1 the average growth of all nine smut cultures in each of the respective nutrient solutions is graphically represented. The data on total growth rather than on the average growth of each smut culture in the respective solution bear out the same conclusion. Such a comparison could have been graphed had it not been for the inability to obtain the results of growth of smut No. 3–2 in the control solution (Table 4). This culture was too viscous to centrifuge quantitatively.

In order to determine further the value of nutrient solution No. 7, the latter was used in a second comparative test with carrot decoction. The test was made under conditions similar to those of the

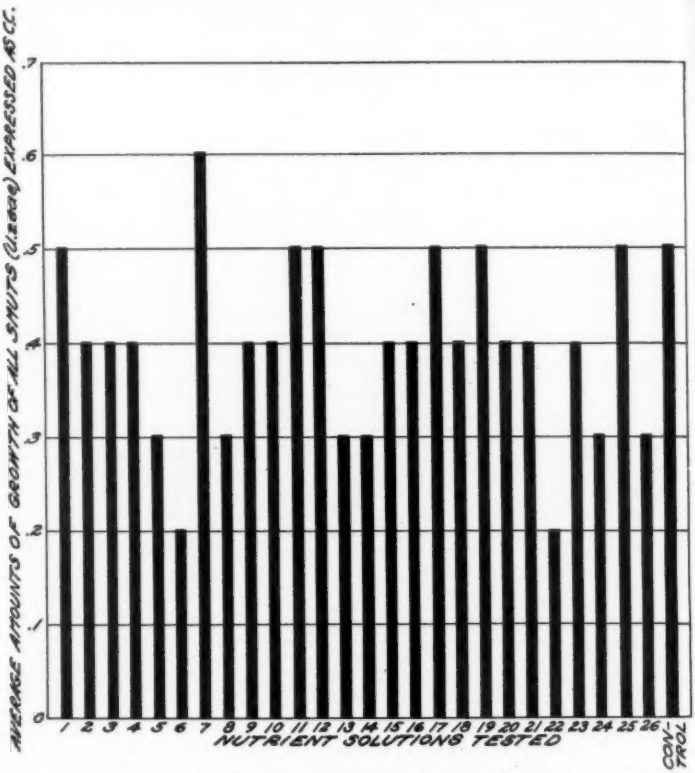

FIGURE 1.—Average growth of 9 collections of corn smut (*Ustilago zeae*) cultured in the control solution (carrot decoction) and in the 26 synthetic nutrient solutions tested. These are averages of totals and necessarily differ somewhat from similar averages calculated from the data of Table 4

first test, except that seven instead of nine cultures of *Ustilago zeae* were used. The results are presented in Table 5.

TABLE 5.—*Average growth of cultures of Ustilago zeae in the control (carrot decoction) and in synthetic nutrient solution No. 7*

[Each figure, which is the average of five cultures, indicates the cubic centimeters of fungus growth precipitated by centrifugation for three minutes at 1,800 r. p. m. The incubation period was three weeks]

Nutrient solution	Average fungus growth produced by cultures of the indicated number [a]						
	1ss	2ss	4ss–2	35ss	34–2	50–1	74–2
No. 7	0.5	0.6	1.7	0.8	0.3	0.2	0.2
Control	.5	.5	1.1	.4	.2	.2	.1

[a] Smut cultures Nos. 3–2 and 60–2 are not included because they did not survive and were not available when the work was repeated.

Table 5 shows that the fungus growth of two of the cultures was the same in solution No. 7 and in carrot decoction and that the growth of five of the cultures was greater in nutrient solution No. 7 than in carrot decoction. This confirms the data of Table 4 in showing the value of nutrient solution No. 7.

OPTIMUM DEXTROSE CONTENT

It will be noted that all the nutrient solutions tested, except the control, contained uniformly 10 gm. of dextrose per liter of solution. As this quantity of dextrose was chosen arbitrarily, the desirability of determining the optimum quantity of dextrose is apparent.

As solution No. 7 had proved to be the one best suited for culturing the organism, it was used as the culture medium in tests for this purpose. The dextrose content was varied from 2.5 to 30 gm. per liter, forming 10 nutrient solutions as indicated in Table 6. Flasks containing 50 c. c. of each solution were used, as previously described. Each 50 c. c. lot was inoculated with one drop of a carrot-decoction culture of smut No. 4ss–2, generally the most rapidly growing culture in the group, but for some unknown reason not always constant in its growth responses under supposedly identical conditions. The contained cultures were incubated at a temperature of 25° ± 0.5° C., and twice each day the flasks were shaken to prevent colony formation. At the end of 3, 6, and 9 weeks, respectively, the quantity of growth in the three sets of five flasks of each solution was determined by centrifugation as previously described. The results are given in Table 6 as averages of the five flasks of each series.

TABLE 6.—*Results of tests to determine the optimum amount of dextrose to use in synthetic nutrient solution No. 7 for culturing Ustilago zeae*

[Each figure indicates the cubic centimeter of fungus growth precipitated by centrifugation for three minutes at 1,800 r. p. m.]

Culture solution No.	Dextrose (grams per liter)	Average growth of smut culture No. 4ss–2, after indicated period			Culture solution No.	Dextrose (grams per liter)	Average growth of smut culture No. 4ss–2, after indicated period		
		3 weeks	6 weeks	9 weeks			3 weeks	6 weeks	9 weeks
7–a	2.5	1.1	1.0	1.0	7–e	15.0	0.8	0.7	1.0
7–b	5.0	1.3	1.7	1.9	7–f	17.5	.7	1.0	1.0
7–c	7.5	1.1	1.9	2.0	7–g	20.0	.6	.8	.8
7	10.0	1.0	1.7	1.8	7–h	25.0	.5	.6	.5
7–d	12.5	.9	1.1	1.5	7–i	30.0	.5	.5	.5

From the data of Table 6 it is evident that growth is definitely limited by the addition of less than 5 gm. or more than 10 gm. of dextrose per liter of culture solution. The optimum quantity appears to vary from 5 to 10 gm. of dextrose per liter, depending upon the length of time the culture is incubated.

COMPARISON OF DEXTROSE WITH MALTOSE

Using maltose, Sartoris [4] found that the optimum concentration for culturing *Ustilago zeae* and certain other species of the same genus was M/7, as shown in his Table 5. He used this concentration (M/7)

[4] SARTORIS, G. B. Op. cit.

in a study of the relative influence of dextrose, lactose, levulose, and maltose on the growth of certain smut species, including *U. zeae*. A solution of M/7 concentration would correspond to approximately 51.5 gm. per liter for a disaccharide ($C_{12}H_{22}O_{11}.H_2O$) and to approximately 28.3 gm. per liter for a monosaccharide ($C_6H_{12}O_6.H_2O$). This concentration (28.3 gm. per liter) of dextrose used by Sartoris is from 2.8 to 5.7 times as concentrated as the optimum concentration (5 to 10 gm. per liter) found by the writer in growing corn-smut culture No. 4ss–2. The results obtained by the writer can not be compared directly with those obtained with *U. zeae* by Sartoris (his Tables 5 and 6) because his data are not reported in quantitative units of measure. The quantities of growth obtained in his experiments were determined, apparently, by observation and reported according to arbitrarily chosen scales, the minimum of which was indicated by the figure 1 and the maxima of which were indicated by the figures 4 or 5.

Sartoris [5] found that maltose was the best sugar for the development of secondary spores of *Ustilago zeae*. As dextrose had been used as the source of carbohydrate throughout these experiments, it was decided to compare the growth of smuts in nutrient solution No. 7 containing dextrose with the growth of smuts in the same solution in which maltose had been substituted for the dextrose. Fifty cubic centimeters of each solution was used per 125 c. c. flask, and triplicate cultures for all the smuts used previously were made in each of the two solutions. The cultures were incubated and shaken as indicated in previous experiments. After three weeks the quantities of growth were determined by centrifugation and are expressed as cubic centimeters of smut thrown down. These data are given in Table 7.

TABLE 7.—*Average growth of seven smut cultures (Ustilago zeae), each incubated three weeks in five flasks of nutrient solution No. 7, containing either dextrose or maltose at the rate of 10 grams per liter*

[Each figure indicates the cubic centimeters of fungus growth precipitated by centrifugation for three minutes at 1,800 r. p. m.]

Nutrient solution No. 7 containing—	Average growth of culture No.						
	1ss	2ss	4ss–2	35ss	34–2	50–1	74–2
Dextrose	0.6	0.7	1.6	0.7	0.5	0.3	0.2
Maltose	1.1	.8	2.0	1.0	.6	.2	.2

The data of Table 7 indicate that with five of the smut cultures maltose supported more growth than did dextrose. In one case the quantity of growth was the same, and in one case dextrose supported more growth. These data indicate also the variable carbohydrate metabolism of the various smut cultures, the variation being most common in those cultures each of which was derived from a single sporidium (Nos. 1ss, 2ss, 4ss–2, and 35ss).

[5] SARTORIS, G. B. Op. cit.

CONCLUSIONS AND SUMMARY

The various nutrient media (decoctions, extracts, or infusions of plant parts or plant products) generally used for culturing *Ustilago zeae* are unsatisfactory. This is true especially in corroborative work or repeated experiments leading to the possible segregation, classification, and identification of the various physiologic forms of *U. zeae*. It is no less true in experiments dealing with the fundamental nature of resistance of corn to infection by *U. zeae*. There is need, therefore, for a synthetic nutrient medium composed only of definite, purified, chemical compounds, thus permitting accurate duplication at any time and in any laboratory.

The data reported show that synthetic nutrient solution No. 7, described herein, meets this need and satisfies the requirements for such a medium. Experiments with nine separate smut cultures (*Ustilago zeae*) have demonstrated its superiority over carrot decoction and the other solutions tested. Nutrient solution No. 7 is composed of but four inorganic compounds and dextrose in solution, to which agar may be added to produce a solid medium. When these constituents are added in solution there is practically no precipitate formed and the resulting hydrogen-ion concentration is favorable to growth. This situation makes it possible to keep the total concentration of the solution at a constant level and permits accurate duplication, as neither acid nor alkali need be added to adjust the reaction of the solution. The reaction of this nutrient solution before sterilization is pH 7.4, and after sterilization it is pH 5.6.

Synthetic nutrient solution No. 7 has the following composition, expressed as grams per liter of solution:

K_2SO_4	0.3 gm.
NH_4NO_3	.1 gm.
$CaCl_2$	.1 gm.
$Mg_3(PO_4)_2 \cdot 4H_2O$	.1 gm.
Dextrose	10.0 gm.
Distilled water to make	1.0 liter

When used as a solid medium, 1.5 per cent of agar was added. For certain physiologic forms of smut (*Ustilago zeae*), it may be desirable to substitute maltose for dextrose. Maltose, however, is from 10 to 15 times as expensive as dextrose.

15141—30——2

PREDACIOUS NEMAS OF THE GENUS NYGOLAIMUS AND A NEW GENUS, SECTONEMA[1]

By GERALD THORNE

Associate Nematologist, Office of Nematology, Bureau of Plant Industry, United States Department of Agriculture[2]

INTRODUCTION

The major nemic pests of cultivated crops in this country have no important natural enemies, at least so far as is known at the present time. This seems to be especially true of the sugar-beet nematode, *Heterodera schachtii* Schmidt, which is one of the most destructive parasites of the sugar beet in the Western States. Many billions of these nemas frequently are found in an acre of ground, and it would seem that such enormous numbers would attract some natural enemies. Predacious nemas of the genus Mononchus occasionally prey upon them, but so far as is known, not to a degree that is of economic importance (*8*).[3]

Among the other predacious species commonly found in these infested fields are those belonging to the genus Nygolaimus Cobb (*1*, *2*) and Sectonema, nov. gen. These two groups were the subject of the researches here recorded.

NYGOLAIMUS

HABITS

FOOD HABITS

For this study fields were selected that were inhabited by several species of nygolaims and heavily infested with *Heterodera schachtii*. Soil samples were collected from about the beet roots and the nemas secured by sifting and gravity methods. Many nygolaims were found living in close proximity to the females of *H. schachtii* that were clinging to the beet roots. These females apparently offered ideal victims for the nygolaims, for, being attached to the beet roots, they could not escape. Hundreds of specimens were collected and the intestines carefully examined for traces of the body contents of *H. schachtii* or other nemas, but in not a single instance were such remains found.

In many of the specimens examined it was possible to identify the setae and cuticle of oligochaete worms (Enchytraeidae), and practically all individuals contained material that appeared to be the body contents of these worms. This was especially true of small species like *Nygolaimus vulgaris*, which are too small to swallow anything except the liquid or granular contents of the worm's body.

When fed in the laboratory both nygolaims and sectonemas invariably refused to devour other nemas but frequently attacked and fed

[1] Received for publication Feb. 21, 1930; issued October, 1930.

[2] The writer is deeply indebted to Dr. N. A. Cobb and Dr. G. Steiner, of the Office of Nematology, for helpful criticisms and suggestions during the preparation of this paper. Thanks are also due Dr. J. G. de Man, of Ierseke, Netherlands, for verifying the identification of *Nygolaimus brachyurus* and *N. hartingii*.

[3] Reference is made by number (italic) to Literature Cited, p. 465.

upon the oligochaetes. In this respect *Sectonema ventralis* was found to be more voracious than any of the nygolaims.

Method of Attack

The nygolaims apparently located the oligochaete by touch only, or at least by very close proximity. Often the head of the nygolaim may pass very near the worm without the nygolaim's appearing to observe its presence. When the nygolaim locates the oligochaete it presses its lips tightly against the body, the lips probably being held there by the suction of the esophagus. The pharynx is then thrust forward, as shown in Figure 8, A, B, C, and the tooth driven into the body of the victim. The tooth is then withdrawn and the body contents of the prey sucked out. The writer has not observed a feeding nygolaim devour the skin of a victim, but that this frequently is done, at least by the larger species, is shown by the many specimens that contain the setae and cuticle of oligochaetes in the intestine.

From the knowledge at present available it appears that the nygolaims are not of economic importance in the control of plant-infesting nemas.

Habitat

The nygolaims inhabit both soil and fresh water, but at present those in the soil appear much the more numerous. Like the predacious mononchs they prefer the lighter sandy soils, probably for the reason that sandy soils give more freedom of movement for pursuit and capture of their prey. Occasionally, however, colonies of the smaller species are observed in clay loams. Some species are found only where there is an abundance of moisture, while others prefer the arid desert soils in which activity is limited to only a few weeks in the spring and fall when the soil is moistened by rains. Apparently there is only one generation each year, most of the eggs being deposited during April, May, and June, although rarely gravid females are found during other months.

The distribution of the various species in a locality is usually quite definite. Some are found in the cultivated valley soils, while others appear only in collections from the mountains. The largest populations usually occur in the cultivated fields.

In no instance has the same species been found inhabiting both cultivated and virgin soil. The native species disappear when the land is cleared and irrigated, and in their place are found species whose origin is problematical. Near Salem, Utah, *Nygolaimus tenuis* and *N. vulgaris* are numerous in cultivated fields, while in virgin soil only a few rods distant *N. brachyurus* and *N. bisexualis* are the species found.

Nygolaimus vulgaris, *Sectonema ventralis*, and Oligochaeta were present in the two fields located near Lewiston and Salem, Utah, where the studies were made on the mononch populations (*8*). *N. tenuis* was also present in the Salem field, while *N. brachyurus* and *N. amphigonicus* were studied in virgin soil near this field. The vertical range of habitat for these species is given in Table 1. The numbers of individuals are the totals counted for each species and are not comparable as to the relative numbers actually present in the soil, because only two $\frac{1}{1000000}$-acre samples were examined for *N. brachyurus* and *N. amphigonicus*, while 34 samples were examined for *N. vulgaris* and 30 for *N. tenuis*.

TABLE 1.—*Vertical range in the soil of Nygolaimus (four species) Sectonema ventralis, and Oligochaeta*

Depth	Nygolaimus vulgaris	N. tenuis	N. amphigonicus	N. brachyurus	Sectonema ventralis	Oligochaeta
Inches	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
3-2	721		7		2	197
1-4	922		4	11	24	420
5-6	516		1	10	20	439
7-8	235			6	19	407
9-10	142	1		4	12	196
11-12	80	22		2	6	134
13-14	48	14			13	64
15-16	2	12			2	64
17-18	5	5			7	64
19-20	4	7			1	37
21-22		4			1	34
23-24		2				20
Total	2,675	67	12	33	107	2,076

The population per acre for the following species was computed from representative soil samples; *Nygolaimus vulgaris*, 78,888,000; *N. tenuis*, 2,250,000; *N. amphigonicus*, 6,000,000; *N. brachyurus*, 16,500,000; *Sectonema ventralis*, 6,727,000; Oligochaeta, 107,700,000.

It is evident that all these species, except *Nygolaimus tenuis*, generally are found in the first foot of soil, as is common with most nemas. Unfortunately, no data are available on other species collected by the writer, but these are from mountain or desert soil from which the samples usually were taken to a depth of less than 1 foot; so it may be concluded that, of the species studied, *N. tenuis* is the only one that favors a deep habitat. In no instance has there been observed any tendency for a species to vary its depth of habitat in response to changes in moisture or temperature. The above data are composite results of many samples taken at all months of the year, and include only a small portion of the individuals of these species that actually have been observed in the extensive soil-examination work carried on by the writer.

GENERAL STRUCTURE

In general appearance certain nygolaims so closely resemble nemas of the genus Dorylaimus that various nematologists have placed them in that genus without noting their true identity.

While the writer was examining a collection of what was supposed to be *Dorylaimus hartingii* his attention was attracted by the three cardiac glands and the dorsal organs crowding the anterior end of the intestine. These were so unusual in Dorylaimus that a more careful examination of the specimens was made, and it was found that the tooth was located on the left submedian wall of the pharynx. This immediately placed the species in Nygolaimus, and a careful search through the writer's collection of about 90 species of dorylaims revealed 10 more species of Nygolaimus that previously had been overlooked.

During this investigation these two closely related genera were found to differ in the following respects:

Nygolaimus	*Dorylaimus*
Tooth set on left submedian wall of pharynx.	Tooth submedian, but not so distinctly pharyngeal.
No definite guiding ring for tooth.	Definite guiding ring for tooth present.
Food does not pass through tooth.	Food passes through tooth.
Pharynx deep and definitely set off.	Pharynx not deep or definite.
Three conspicuous glands at base of esophagus.	Glands not conspicuous at base of esophagus.
Lips of vulva not cuticularized.	Lips of vulva cuticularized.
Gubernaculum posterior to the spicula present.	Gubernaculum posterior to the spicula not present.

BODY.—Nygolaims are comparatively large nemas, the length varying from slightly over 1 mm. in *Nygolaimus dubius* to 4–5 mm. in *N. denticulatus* and *N. ferox*. The body is slender, the width rarely exceeding 2.5 per cent of the length and often being less. The neck is slightly convex conoid to the head. Posteriorly the body begins to taper some distance in front of the anus, beginning to decrease suddenly near the anus and ending in a hemispherical to elongate-conoid tail without a spinneret.

PORES.—The series of lateral pores described by Cobb for *Nygolaimus denticulatus* doubtless is present in all species. In some of them, especially the smaller ones, they are extremely difficult to see, or even invisible. The anterior pore of the lateral series sometimes is located in the cuticle near the center of the escutcheon-shaped amphid. The first three or four pores lie almost in line, then the series begins to diverge into two distinct lines that continue nearly to the terminus. On *N. brachyurus* and others there are dorsal and ventral series reaching from the head nearly to the middle of the esophagus.

The phasmids apparently are not definite but are included in the lateral series of pores. However, there usually is one pore near the middle of the tail that is more prominent than the others, and this may be the phasmid proper.

HEAD.—The six confluent lips are amalgamated into a hexagonal mass bearing two circlets of innervated papillae that do not interfere with the contour of the lips. The inner circlet consists of 6 papillae. The outer circlet has only 6 in small species, but in the larger forms like *Nygolaimus brachyurus* and *N. vorax* there are 10. From a front view the lips appear to lack lip flaps, and the entrance to the pharynx is closed by drawing the lips together. The broad amphids are escutcheon-shaped with obscure slitlike openings. Frequently the body twists until the amphids, seen dorsoventrally, give the head the appearance of being set off by a deep constriction, whereas in reality it may not be set off in any manner.

PHARYNX.—The pharynx opens through a narrow vestibule made up of six folds that allow for great expansion. (Fig. 7, B.) From the usual lateral view these plications frequently appear to form a guiding ring for the spear. The pharynx proper is of double structure, the anterior portion being somewhat fusiform and the posterior more or less tubular. The anterior portion is made up of three sections arranged tandem, the first extending from the vestibule and widening out to the full width of the pharynx. The second is hexagonal in structure and forms the main body of the pharynx. (Fig. 7, C.) The third portion is pentagonal, due to the fusing of the two left submedian plates into a solid mass to support the tooth. (Fig. 7, D.) This third section narrows to join the long, slightly triquetrous, tubular posterior

portion of the pharynx. These sections form the eversible portion of the pharynx and allow the tooth to be thrust forward out of the mouth as shown in Figure 8, A–C.

Tooth.—The tooth of *Nygolaimus denticulatus* and *N. menzelii* tends to be quite arcuate, but in the remainder of the species it is almost straight and projects directly forward from its location on the wall of the pharynx. Usually it is hollow almost the entire length, but in *N. vulgaris* and *N. bisexualis* the distal portion is an exceedingly slender, solid point.

Esophagus.—This organ is quite typically dorylaimoid. Usually the posterior three-fifths is enlarged by a gradual expansion, and frequently the enlarged portion is irregular in width. (Fig. 9.) The musculature is strongly developed and internal glands are present.

Cardiac glands.—These are one of the distinctive features of nygolaims, and it is to be wondered at that they have not been noted by previous investigators. They closely resemble the glands of Trilobus in their size and position, one being ventral, the other two subdorsal. Their secretions enter the digestive tract through pores located at the junction of the esophagus and the intestine. (Fig. 3, C.)

A pair of conspicuous organs are present in *Nygolaimus teres, N. dubius, N. acuticaudatus,* and *N. hartingii.* These are located subdorsally and crowd the anterior end of the intestine. (Figs. 12, D; 13, D; and 15, D.) Their function is problematical, but from the fact that ampullae extend forward from them and enter into large cells located in the lateral chords, it appears evident that they produce secretions. Their presence was noted by De Man in his original description of *N. hartingii.* The size of these organs varies considerably in individuals from the same collection, in some being less than the body width in length, in others four or five times as long as the body width.

Digestive tract.—The intestine is always distinctly set off from the esophagus and is made up of cells that require 6 to 10 for a circumference. The cells usually are well filled with rather uniform fine granules that often are light brown in color, frequently giving the body a rich brown appearance. The walls of the intestine are thin and allow observation of the contents of the lumen. The prerectum is short, usually being one to three times as long as the rectum. The rectum is from one to one and one-half times as long as the anal body diameter and ends in a conspicuous depressed anus with distinct lips.

Reproductive systems.—Males are known of only 7 of the 16 species of Nygolaimus. That the females of at least some of the other species are syngonic may safely be predicted, since large collections made at various times of the year have invariably failed to contain males.

Two ovaries are present in all the known species. The gonads are of approximately the same length and together rarely occupy more than one-fourth of the body length. The ovaries are always reflexed.

There are two testes, of which the left is the longer and extends forward about half the length of the body and does not have the distal end reflexed. The right is the shorter of the two and is reflexed a distance equal to three or four times the width of the body. The testes contain several hundred spermatozoa of fairly large size. The

spicula are thick, strong, and slightly arcuate. They rest on short, thick gubernacula that sometimes are difficult to see because of their peculiar refractive index. Bifurcate lateral guiding pieces usually are present and are joined to the gubernacula by bands of muscles. The musculature of the male tail is not so heavily developed as in some genera. The series of male supplementary organs is variable, only a single pair of anal papillae being present in *Nygolaimus brachyurus*, while in *N. teres* and others there are as many as eight preanal supplements in addition to the pair of papillae just in front of the anus.

TAXONOMY

There follows a key to the species of Nygolaimus, together with descriptions of the 6 forms previously known and 10 hitherto undescribed species. These fall into two distinct groups, for which the subgeneric names Nygolaimus and Nygolaimium are here proposed. The various species are shown in Figures 1 to 17. All figures of heads are shown at a magnification of 1,000, while the tails and other parts of the body are shown at 500 unless otherwise stated.

KEY TO THE GENUS NYGOLAIMUS

	Subgenera and species	Figure No.
Pharynx without denticles:		
Type *N. pachydermatus* Cobb (*1*), 1913	NYGOLAIMUS	
Terminus blunt and rounded.		
Lip region set off by a constriction.		
Tooth hollow less than half its length.		
Cuticle of tail with distinct outer layer	*vulgaris*, n. sp.	1
Cuticle of tail without distinct outer layer	*bisexualis*, n. sp.	2
Tooth hollow almost to apex.		
Length rarely over 2 mm.		
Lip region only one-third as high as wide	*pachydermatus* Cobb (*1*), 1913	5
Lip region one-half or more as high as wide		
Pharynx three times as deep as lip width	*tenuis*, n. sp.	3
Pharynx twice as deep as lip width	*amphigonicus*, n. sp.	4
Length well over 2 mm.		
Tail hemispherical	*obtusus*, n. sp.	6
Tail not hemispherical, more conoid	*brachyurus* De Man (*4*), 1884	7
Lip region continuous with head contour.		
Length under 2 mm	*teres*, n. sp.	12
Length over 2 mm.		
Pharynx about four times as deep as lip width	*aquaticus*, n. sp.	8, 9
Pharynx less than three times as deep as lip width.		
Tail shorter than anal body diameter	*ferox*, n. sp.	10
Tail as long as anal body diameter	*shadini* Filipjev (*3*), 1928	11
Terminus acute or subacute.		
Tooth half as long as lip width	*acuticaudatus*, n. sp.	13
Tooth about as long as lip width.		
Tail twice length of anal body diameter	*dubius*, n. sp.	14
Tail three times anal body diameter	*hartingii* De Man (*4*), 1884	15

KEY TO THE GENUS NYGOLAIMUS—Continued

Pharynx with area bearing minute denticles:	Subgenera and species	Figure No.
Type *N. denticulatus* Cobb (*2*), 1922	NYGOLAIMIUM	
Length under 3 mm	*menzeli* Micol. (*5*), 1925.	16
Length 4 mm. or more	*denticulatus* Cobb (*2*), 1922	17

TECHNICAL DESCRIPTION OF SPECIES

Nygolaimus vulgaris, n. sp. (Fig. 1.)

Diagnosis:[4]

			12.0	12.0		
3.0	8.0	29.0	52.0		98.1	1.1–1.8 mm.
1.2	2.1	2.5	2.7		1.5	

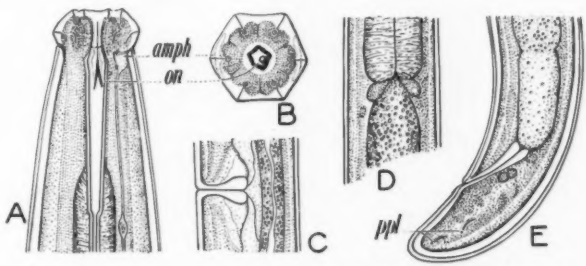

FIGURE 1.—*Nygolaimus vulgaris,* n. sp.: A, Head, slightly subdorsal view; *amph,* amphid; *on,* tooth; B, face view showing arrangement of head papillae, also cross section of tooth and third section of pharynx; C, vulvar region; D, cardiac region; E, tail; *ppl,* caudal papillae

Cuticle with a distinct outer layer which is especially conspicuous on the tail. Spear very slender, three-fourths as long as the head width and hollow only one-third its length. Ovaries reflexed four-fifths their length. Males unknown from among several thousand specimens collected in all months of the year.

General characters.—Body assuming the form of an open letter C, especially when killed by gradual heat. Transverse striae indistinct. External amphids half as wide as the head. Second and third sections of pharynx obscure. Posterior portion of esophagus one-half as wide as the neck. Intestine one-half as wide as the body, its cells containing minute, colorless granules. Lateral chords one-seventh as wide as body.

The most prevalent nygolaim of the Western States. Collected from cultivated valley soils of Utah, Idaho, California, Colorado, and Oregon. See Table 1 for depth distribution.

Nygolaimus bisexualis, n. sp. (Fig. 2.)

Diagnosis:

			10.0	10.0		
1.9	8.0	27.0	52.0		98.2	1.5–2.0 mm.
0.8	1.8	2.0	2.2		1.3	
2.0	9.6	27.0	M		98.3	1.7 mm.
0.8	1.8	1.9	1.9		1.4	

Cuticle without a distinct outer layer as in *N. vulgaris.* Striae resolvable into rows of excessively minute dots. Spear very slender, as long as the width of the lips. Lateral chords one-fifth to one-third as wide as the body. Cardiac glands large and conspicuous. Ovaries reflexed only half their length. Males about equal in number to females.

[4] The "Diagnosis" includes the formula and the first paragraph following. The remaining paragraphs give general, not necessarily specific, information.

General characters.—Intestine less than half as wide as the body, its cells filled with many dark granules that frequently give a slightly tessellated appearance. The last two pores of the lateral series usually are easily seen on the tail, but the remainder of the series are indistinct. Both sexes assume the form of an open letter C when killed by heat.

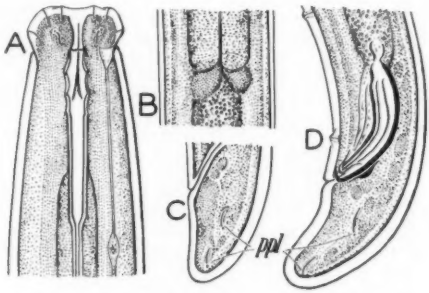

FIGURE 2.—*Nygolaimus biserualis*, n. sp.: A, Head; B, cardiac region; C, female tail; *ppl*, caudal papillae; D, male tail

Spicula slightly arcuate with lateral guiding pieces and resting upon small gubernacula. A pair of papillae lie side by side just in front of the anus. About one and one-half body widths in front of these there usually is a ventral supplement.

Quite common in soils from canyons near Salt Lake City, Utah.

Nygolaimus tenuis, n. sp. (Fig. 3.)

Diagnosis:

			10.0	10.0		
1.7	6.0	22.0	52.0		98.9	2.0 mm.
0.6	1.1	1·4	1.5		.9	

Spear only about half as long as the width of the lip region. Pharynx almost three times as deep as the width of the lips. Lateral chords only one-twelfth to one-tenth as wide as the body. Posterior portion of body not arcuate.

General characters.—The body twists more than in any other known nygolaim, frequently making half a turn or even more. Cardiac glands unusually large.

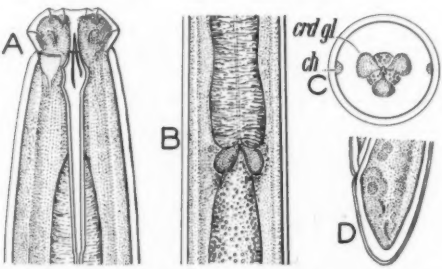

FIGURE 3. *Nygolaimus tenuis*, n. sp.: A, Head, slightly subventral view; B, cardiac region; C, cross section through cardiac region; *crd gl*, cardiac glands; *ch*, lateral chord; D, tail

Intestine narrow and tapering at the anterior end except when gorged with food. Ovaries reflexed half their length. Rectum and prerectum each about as long as the tail. Males unknown among about 125 specimens.

Usually found in small numbers rather deep in sandy loam valley soils of Utah. See Table 1 for depth distribution.

Nygolaimus amphigonicus, n. sp. (Fig. 4.)

Diagnosis:

			9.0 9.0		
1.8	7.5	23.0	54.0	98.5	1.9 mm.
0.9	1.5	1.7	1.8	1.2	
1.7	8.0	26.0	M	98.1	1.53 mm.
0.9	1.5	1.9	1.9	1.5	

Similar to *Nygolaimus tenuis*, but with these differences: Pharynx only about twice as deep as the width of the lips. Striæ very fine but under favorable conditions are resolvable into dotlike markings. Lateral chords one-fifth to one-fourth as wide as the body. Males about as numerous as females.

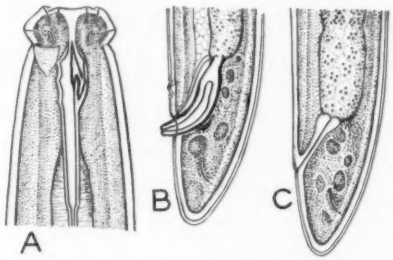

FIGURE 4.—*Nygolaimus amphigonicus*, n. sp.: A, Head, slightly submedial view, with tooth not yet completely joined to the pharyngeal wall after the last molt; B, male tail; C, female tail

General characters.—Cardiac glands not conspicuously large. Both sexes assume an almost straight position when killed by gradual heat. Ovaries reflexed half their length, the front being on the right, the rear on the left, side of the body. Intestine three-fourths as wide as the body, its cells containing a few small, very dark, scattered granules.

From about roots of tomato plants, Buena Park, Calif., and mountain and desert soil from St. George, Zion Canyon, Salem, and Jensen, Utah.

Nygolaimus pachydermatus Cobb, 1915. (Fig. 5.)

0.6	7.7	32.0	66.0	98.0	1.8 mm.
1.1	1.9	2.4	2.5	1.9	

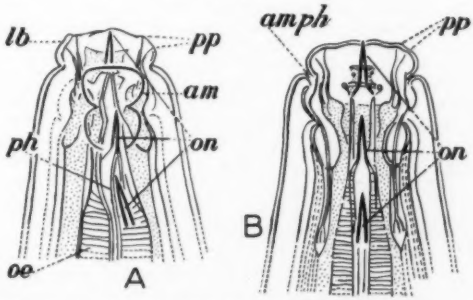

FIGURE 5.—*Nygolaimus pachydermatus* Cobb: A, Head of molting specimen; *am*, amphid; *lb*, lips; *oe*, esophagus; *on*, tooth; *ph*, pharynx; *pp*, papillae; B, head, dorsoventral view; *amph*, amphid; *on*, tooth; *pp*, papillae. (After Cobb)

Measurements derived from a young specimen. Cuticle thicker on the tail. Lips connate, set off by constriction. Amphids half as wide as the corresponding

part of the head. Spear half as long as head is wide, conical, slender, pointed, toothlike; without guiding ring. Posterior half of the esophagus enlarged to three-fourths the width of the neck. Tail convex-conoid. Fixed in Flemming's solution and examined in glycerin. From the roots of cherry trees, Tokyo, Japan.

Nygolaimus obtusus, n. sp. (Fig. 6.)

Diagnosis:

			11.0	11.0	
1.8	7.5	30.0	60.0	98.6	2.9 mm.
0.7	1.8	2.2	2.2	1.6	

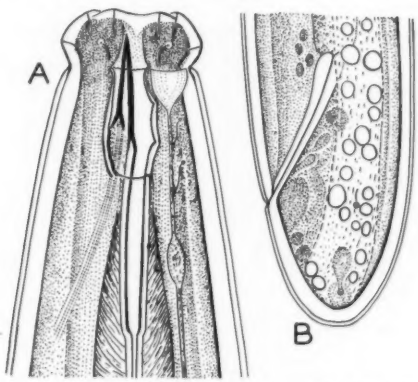

FIGURE 6.—*Nygolaimus obtusus,* n. sp.: A, Head; B, tail

Body but little curved, even in the posterior third. First three sections of pharynx forming a large cavity about one-third as wide as the head and twice as long as wide. Ovaries reflexed one-third their length. Tail, long hemispherical.

General characters.—Lateral chords one-fifth as wide as the body and conspicuous because of the large granules appearing in them. Cardiac glands unusually small. Intestine three-fourths as wide as the body, its cells densely packed with small granules. Anus depressed, rectum one and one-third times as long as the anal body diameter. Prerectum one and one-half times as long as the rectum. The elongate-hemispherical tail bears two pairs of conspicuous papillae, the posterior pair often showing distinct connections with an internal glandlike body. Amphids obscure, about one-third as wide as the head, with inner extensions leading to small sensillas. Behind the amphid and about opposite the base of the vestibule of the pharynx are located two pores with inner connections that apparently end in the tissues of the amphids.

No males found in the single collection of 12 specimens from a sugar-beet field near Ogden, Utah.

Nygolaimus brachyurus De Man. (Fig. 7.)

			9.0	9.0	
1.8	6.0	30.0	57.0	99.1	2.8—3.6 mm.
0.7	1.5	1.6	1.8	1.1	
1.7	6.8	29.5	M	98.9	2.3—2.9 mm.
0.7	1.6	1.8	1.8	1.1	

Several females of this species collected in Utah were sent to De Man, at Ierseke, Netherlands, to verify the determination. He made a careful comparison with the specimens that he had collected in the Netherlands and in Norway, and in a letter dated October 22, 1928, he says:

"The species seems to belong indeed to *Dorylaimus brachyurus* as described in my monograph of 1884. * * * In October, 1913, just 15 years ago, I had the good luck of discovering the male, at that time still unknown, of this species, in dry soil of a meadow in the western part of the Dutch province of North Brabant. This male was 1.55 mm. long, width 1.8%, esophagus 25.0%, tail 1.25%. Besides the usual anal papilla, still another papilla was observed in front of the anus, about twice as far distant from the anus as the length of the tail. Perhaps instead of one preanal papilla there are two submedian papillae placed abreast close by one another. * * * In August, 1918, a female was captured by me in soil covered with moss and grass from a wood in western North Brabant. The length was 3.72 mm., width 2.0%, esophagus 28%, tail 0.9%, vulva at 56%, front ovary 11.3%, rear ovary 11.6%. Another female 4.1 mm. in length was 1.75% wide, esophagus 25%, and tail 0.9%. There were two eggs in each of these females."

From this letter it is evident that the European and American specimens are practically identical in every respect and the species is without doubt a cosmopolitan one.

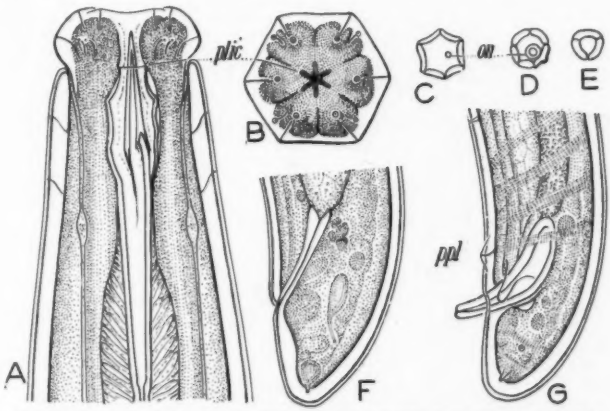

FIGURE 7.—*Nygolaimus brachyurus* De Man: A, Head; *plic*, plications of pharynx; B, face view; C, cross section of pharynx through second portion; *on*, tooth; D, cross section through third portion; E, same through fourth part; F, female tail; G, male tail; *ppl*, preanal papillae

Commenting on the tooth of this species, De Man says: "Spear comparatively feeble; it presented another structure than in other species and seemed to be placed in a chitinous case or tube."

Ten papillae in the outer circlet. (Fig. 7, B.) Lip region one-third to one-half as wide as the base of the neck. Tooth about as long as the width of the lip region. Lateral chords one-eighth to one-sixth as wide as the body. Ovaries reflexed one-half their length. Rectum and prerectum each somewhat longer than the anal body diameter. The esophagus of immature specimens frequently occupies from 35 to 40 per cent of the body length.

In the Western States the males are rare—only about 1 to 50 females. The strong, slightly arcuate spicula rest on small gubernacula and possess lateral guiding pieces. (Fig. 7, G.) A pair of preanal papillae lie just in front of the anus, and two tail lengths in front of these is a rudimentary ventral supplement; occasionally a second is present one tail length in front of the first.

Small numbers of this species are generally distributed in desert, foothill, and mountain soil of Utah, Nevada, Idaho, and Oregon.

Nygolaimus aquaticus, n. sp. (Figs. 8 and 9.)

Diagnosis:

			14.0	14.0	
2.1	6.0	34.5	51.0	98.5	2.5—3.7 mm.
0.8	1.9	2.5	2.7	1.5	
2.0	6.4	25.4	M	98.4	2.9 mm.
0.9	1.8	2.4	2.4	1.6	

Pharynx four times as deep as the width of the lip region. Tails of both sexes blunt and rounded, slightly longer than the anal body diameter. Female prerectum two and one-half times as long as the rectum.

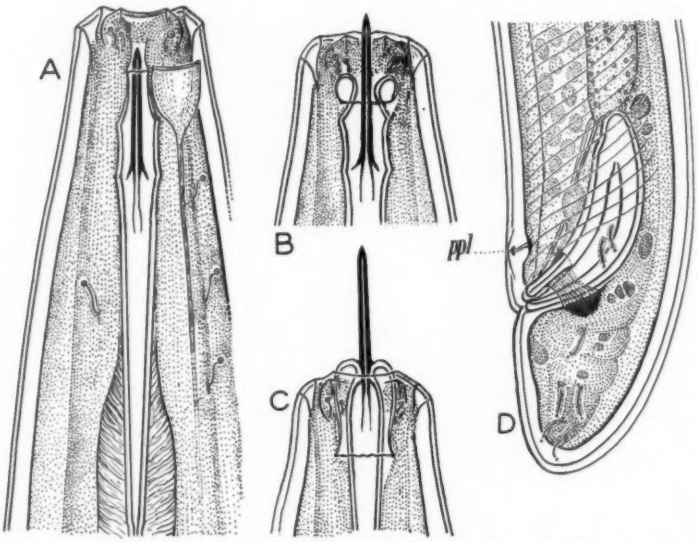

FIGURE 8.—*Nygolaimus aquaticus*, n. sp.: A, Head, slightly submedian view with tooth in place in pharynx; B, same with tooth thrust partly forward and showing the manner in which the pharynx is everted; C, pharynx completely everted; D, male tail; *ppl*, preanal papillae

GENERAL CHARACTERS.—Ten papillae in the outer circlet. Amphids half as wide as the head. Body pores distinct. Enlarged portion of the esophagus usually irregular in width. Cells of the intestine filled with small, brown granules. Rectum as long as the anal body diameter. Ovaries reflexed one-half their length.

Male tail bearing five to eight irregularly spaced, ventral, preanal supplements in addition to the pair of anal papillae. The slightly arcuate spicula are unusually large, rest on a small, somewhat hemispherical gubernaculum, and have furcate, lateral guiding pieces.

Collected from Utah Lake at an elevation of 4,750 feet, and from mountain lakes of Utah at elevations of 8,000 to 10,000 feet. Setae of oligochaetes of the family Naididae were found in several specimens.

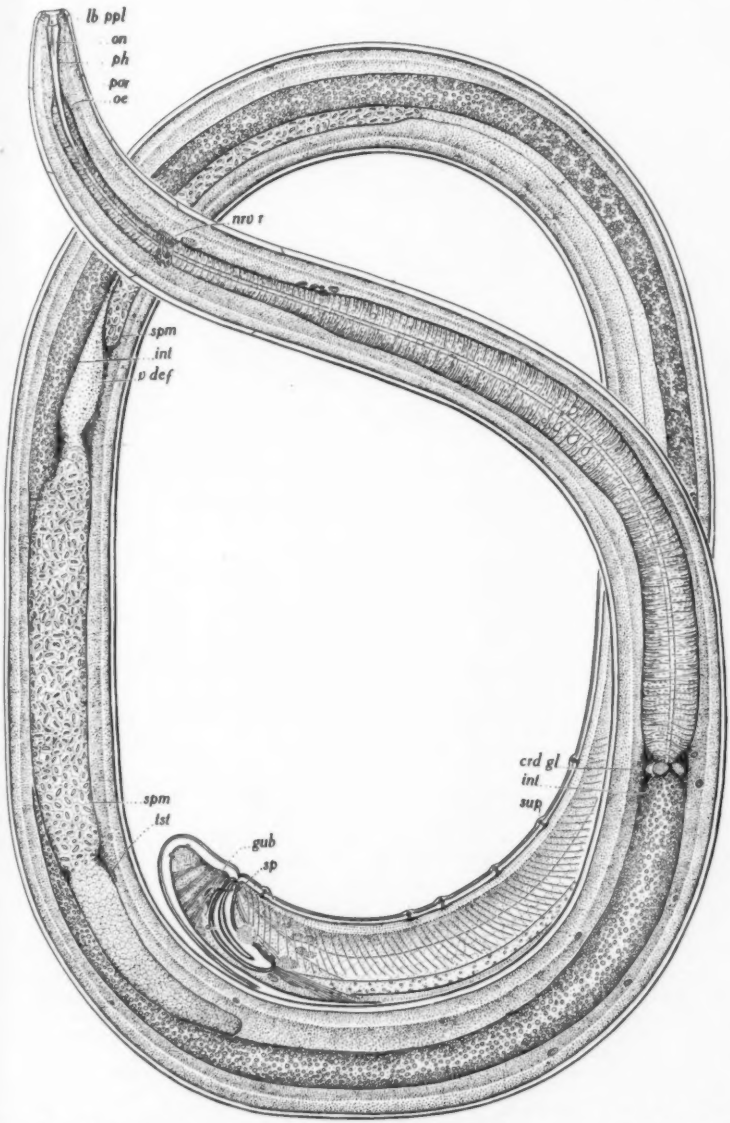

FIGURE 9.—*Nygolaimus aquaticus*, n. sp.: Male; *crd gl*, cardiac glands; *gub*, gubernaculum; *int*, intestine; *lb*, *ppl*, labial papillae; *nrv r*, nerve ring; *oe*, esophagus; *on*, tooth; *ph*, pharynx; *por*, lateral pore; *sp*, spicula; *spm*, spermatozoa; *sup*, supplements; *tst*, testes; *v def*, vas deferens

Nygolaimus ferox, n. sp. (Fig. 10.)

Diagnosis:

			12.0	12.0	
1.8	5.0	15.0	46.0	99.5	3.32 mm.
0.6	1.2	1.6	1.9	0.7	
1.5	4.7	19.0	M	99.0	4.3 mm.
0.5	1.5	1.7	1.7	1.0	

Female tail slightly longer than half the anal body diameter. Esophagus less than 20 per cent of body length. Tooth one-third or one-half as long as the width of the lip region. Gubernacula thin and flat.

General characters.—Ten papillae in the outer circlet. Amphids one-half as wide as the head, conspicuous from a dorsoventral view. Pharynx three times as deep as the width of the lip region. Posterior portion of esophagus half as wide as the neck. Prerectum and rectum of female both slightly longer than the anal body diameter.

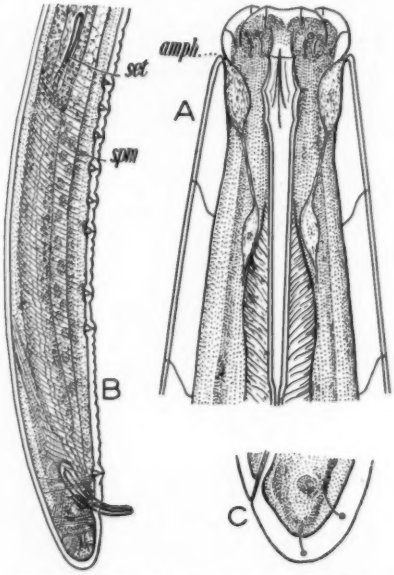

FIGURE 10.—*Nygolaimus ferox*, n. sp.: A, Head, dorsoventral view; *amph*, amphid; B, male tail, × 200; *set*, seta of oligochaete; *spm*, spermatozoa; C, female tail, × 350

Male tail as long as the anal body diameter. The usual pair of papillae lie near the anus, and three tail lengths in front of it are eight irregularly spaced ventral supplements. The thick cuticle between them is marked by creases that give it a wrinkled appearance. The tooth of the male is one-third longer than that of the females in the collection.

Three females and one male from a stream bank, Alsee Mountain, near Corvallis, Oreg. The intestines were gorged with the remains of oligochaetes, including many setae and large pieces of cuticle.

Nygolaimus shadini Filipjev, 1928. (Fig. 11.)

1.6	5.4	20.0	53.0	98.6	3.0–3.71 mm.
0.6	1.7	1.9	2.0	1.1	
1.7	6.2	21.0	M	98.7	3.0–3.67 mm.
0.7	1.5	1.8	1.8	1.1	

Head continuous with body contour. Ten papillae in the outer circlet. Tooth shorter than lip width. Posterior portion of esophagus almost filling the body cavity. Anal muscles conspicuous.

Spicula strong, broad, and arcuate, resting upon a little gubernaculum. Tail with two submedian papillae. A pair of papillae lie just in front of the anus,

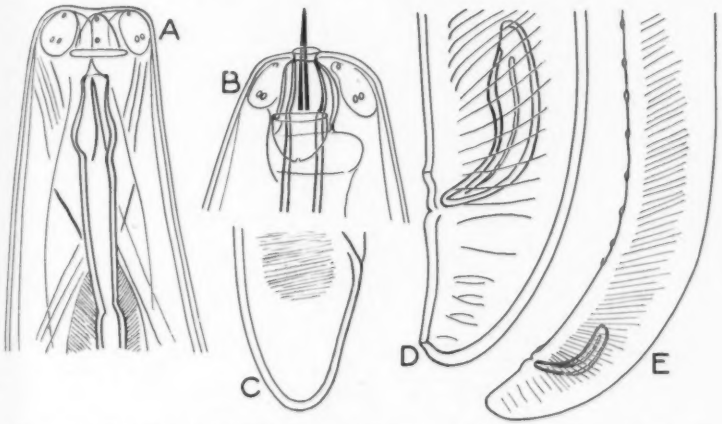

FIGURE 11.—*Nygolaimus shadini* Filipjev.: A, Head; B, lip region with tooth thrust forward; C, female tail; D, male tail; E, posterior portion of male. (After Filipjev)

and about two tail lengths in front of these begins a series of five to seven ventral supplements.

Four males and two females from mud, sand, and sphagnum, collected on the coast at mouth of Klazma River, Oka, District Baliff, Russia (*3*).

Nygolaimus teres, n. sp. (Fig. 12.)

Diagnosis:

			54.0		
2.5	8.0	23.0	M	98.6	1.62 mm.
1.0	1.8	2.1	2.5	1.8	
			15.0	15.0	
2.5	8.0	23.0	50.0	98.4	1.66 mm.
0.8	1.6	2.3	2.6	2.0	

Striae coarser than on any other nygolaim examined and usually can easily be resolved into dotlike markings. Tooth as long as the width of the lip region, hollow almost the entire length. Pharynx three times as deep as the width of the lips. Tail very blunt and rounded. Male with 6 to 10 ventral, preanal supplements in addition to the anal papillae.

General characters.—Lateral chords one-fifth as wide as the body. Ovaries reflexed one-half their length. Prerectum twice as long as the rectum. Lateral series of pores usually visible almost the entire length of the body. A pair of dorsal glands crowding the anterior end of the intestine usually is visible. These

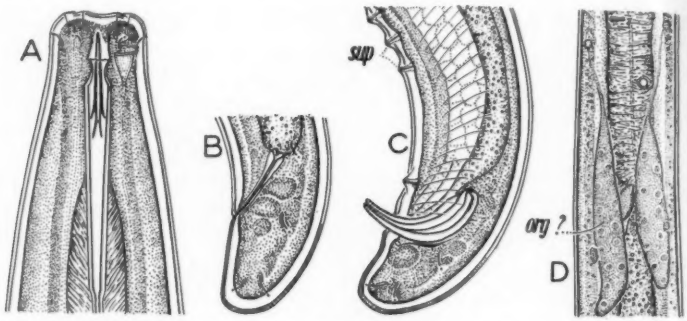

FIGURE 12.—*Nygolaimus teres*, n. sp.: A, Head; B, female tail; C, male tail; *sup*, male supplements; D, cardiac region, *org*, organs of unknown function with their ampullae apparently connecting with cells in the lateral chords

vary greatly in size in different specimens. Males and females about equal in numbers.

Common in foothill and mountain soil in the Wasatch and Uinta Mountains, Utah.

Nygolaimus acuticaudatus, n. sp. (Fig. 13.)

Diagnosis:

		14.0	14.0		
1.9	6.4	20.0	48.0	97.8	1.47 mm.
1.0	2.0	2.0	2.4	1.6	
		60.0			
1.9	6.8	20.7	M	97.6	1.47 mm.
1.0	1.6	2.0	2.2	2.0	

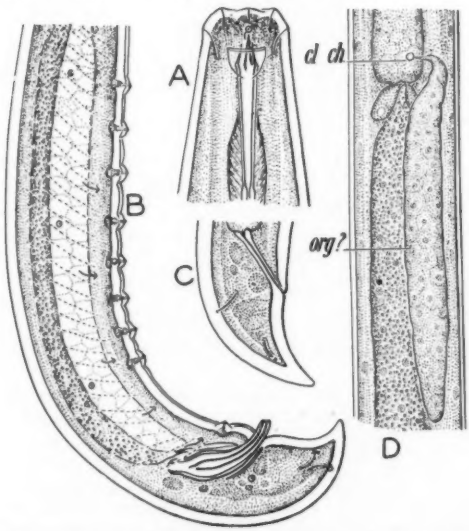

FIGURE 13.—*Nygolaimus acuticaudatus*, n. sp.: A, Head; B, male tail; C, female tail; D, cardiac region; *cl ch*, cell in lateral chord; *org*, organ of unknown function

Lip region continuous with the body contour. Tooth less than half as long as the width of the lip region. Pharynx about twice as deep as the width of the

lips. Tail but little longer than the anal body diameter, ending in a sharp terminus.

General characters.—Amphids obscure slits half as wide as the head. Posterior half of esophagus enlarged. The glandular organs of the cardiac region vary greatly in size, in some specimens being difficult to see, in others attaining a length of two or three body widths. (Fig. 13, D.) Ovaries reflexed one-half their length. Rectum about as long as the anal body diameter. Prerectum three times as long as the rectum.

Male tail bearing a pair of papillae just in front of the anus, and beginning a little more than one body width in front of this pair is a series of 8 to 10 ventral supplements. There are seven pairs of submedian pores. The slightly arcuate spicula rest on slender gubernacula.

A rare species from high mountain soils. This may be the species described by Steiner (*6*) as *Dorylaimus lugdunensis*.

Nygolaimus dubius, n. sp. (Fig. 14.)

Diagnosis:

			12.0	12.0	
3.0	9.0	24.0	46.0	96.8	
1.0	2.1	2.7	2.9	1.6	1.1 mm.

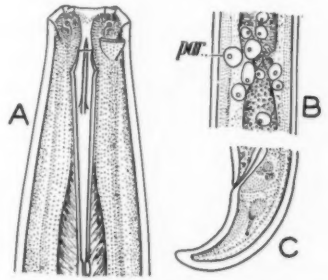

FIGURE 14.—*Nygolaimus dubius*, n. sp.: A, Head; B, cardiac region; *par*, parasitic sporozoans in body cavity; C, tail

Lip region continuous with the body contour. Tooth as long and pharynx three times as deep as the width of the lips. Tail ventrally bent, subacute, and twice as long as the anal body diameter.

General characters.—Posterior half of esophagus enlarged. Cardiac glands and organs crowding the intestine as in *N. hartingii*. Ovaries reflexed two-thirds to four-fifths their length. Rectum as long as the anal body diameter; prerectum two or three times as long as the rectum. No males found among about 40 specimens.

A comparatively rare species from the Wasatch and Uinta Mountains, Utah, at elevations of 10,000 to 12,000 feet.

This species may be identical with *Dorylaimus graciloides* Steiner (*6*).

Nygolaimus hartingii De Man, 1884. (Fig. 15.)

			10.0	10.0	
3.0	7.5	22.0	45.0	95.6	
0.9	2.2	2.4	2.6	1.3	1.3 mm.

Specimens of this species collected in Utah were sent to De Man, and in his letter of October 22, 1928, he states that they apparently are identical with his *Dorylaimus hartingii*. The European specimens are slightly larger, 1.4 to 1.8 mm., but the body proportions are quite similar.

The conoid, subacute, ventrally bent tail is the most distinguishing feature of this species. Head continuous with the body contour. Tooth about as long and pharynx three times as deep as the lip width. Posterior half of the esophagus enlarged but irregular in size. The cardiac glands and organs crowding the intestines are conspicuous, and were figured by De Man in his original description. Ovaries symmetrical, generally reflexed about three-fourths their length. The position of the ovary in the body seems to vary. In one collection

of 22 specimens there were 20 with the front ovary on the left side of the body. In another group of 16 specimens from high mountain soil there were 11 with

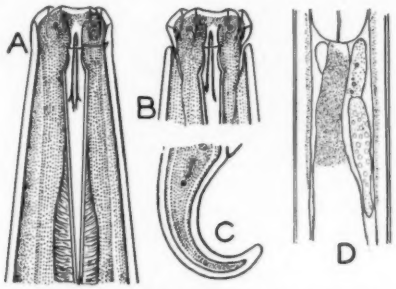

FIGURE 15.—*Nygolaimus hartingii* De Man: A, Head, slightly submedian view; B; lip region, dorsoventral view; C, tail; D, cardiac region. (After De Man)

the front ovary on the left side. Prerectum two to three times as long as the rectum. Males unknown from about 80 specimens.

Collected in a sugar-beet field adjacent to Utah Lake, near Provo, Utah, and at an elevation of 10,500 feet in Bells Canyon, near Draper, Utah.

Nygolaimus menzeli Micoletzky, 1925. (Fig. 16.)

3.5	?	30.0	51.0	98.8	2.7 mm.
0.7	?	3.3	3.5	2.0	

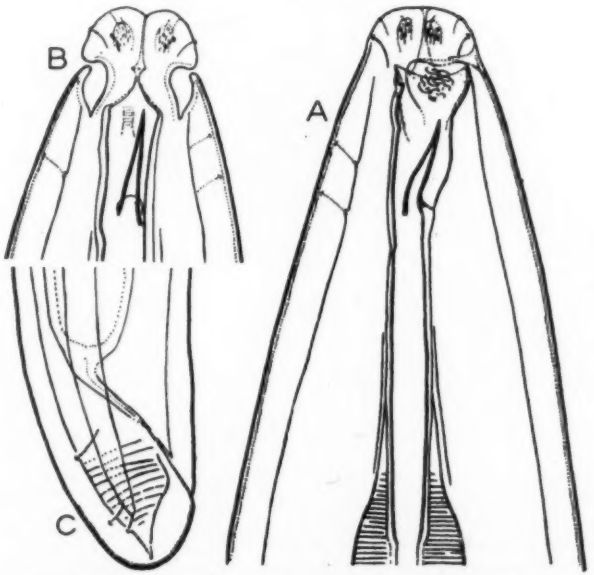

FIGURE 16.—*Nygolaimus menzeli* Micoletzky.: A, Head, lateral view; B, head, dorsoventral view; C, tail. (After Micoletzky (5))

Cuticle reaching a thickness of 6 μ near the head, 4–4½ μ at the middle, and 13–16 μ on the tail. Minute striations on the cuticle at the extremities. Lateral

chords one-seventh the body width. Innervated papillae on the tail and near the head. Anterior end of nema very considerably narrowed. Amphids beaker-shaped with many fibrils. Lips and papillae rudimentary. Pharynx over four times as deep as the width of the lip region. Esophagus at first one-fourth as wide as the body but later expanding to one-half to two-thirds as wide. Ovaries typical; vulva with strong sphincter and radial muscles; vagina reaching halfway across the body.

From Surinam, Dutch Guiana, South America.

Nygolaimus denticulatus Cobb, 1922. (Fig. 17.)

1.8	5.4	22.0	Y	98.8	4.3 mm.
1.1	1.3	1.6	1.6	1.4	

Cuticle thick, transparent, colorless, naked, measuring 5 μ through near the head and 15 μ on the tail. Transverse striae very fine, barely resolvable into rows of excessively faint dots. Extending from near the head to the tail, there is a double series of about 150 sublateral pores not forming exact lines but arranged slightly irregularly. From each pore a narrow tube, 0. 75 μ in diameter,

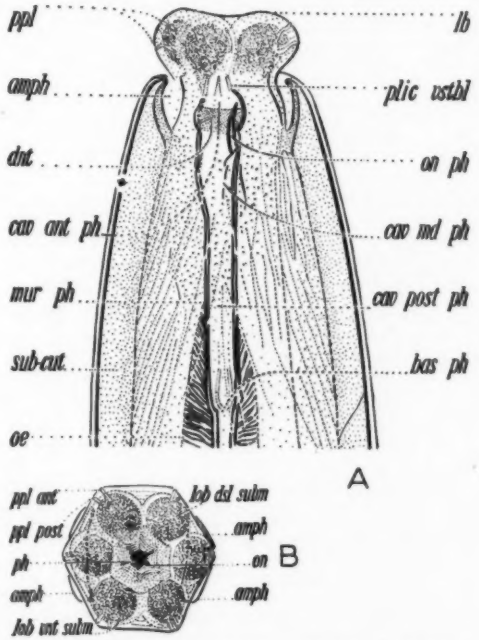

FIGURE 17.—*Nygolaimus denticulatus* Cobb.: A, Head, dorso-ventral view; *amph*, amphid; *bas ph*, base of pharynx; *cav ant ph*, cavity of anterior pharynx; *cav md ph*, cavity of median pharynx; *cav post ph*, cavity of posterior pharynx; *dnt*, denticles; *lb*, lips; *mur ph*, pharynx wall; *oe*, esophagus; *on ph*, tooth on pharynx wall; *ppl*, labial papillae; *sub cut*, sub cuticle; B, face view; *amph*, amphid; *lob dsl subm*, dorsal submedian lobe of lip region; *lob vnt subm*, ventral submedian lobe of lip region; *ppl ant*, papillae of anterior circlet; *ppl post*, papillae of posterior circlet. (After Cobb (2))

extends to the lateral chord, where it rather suddenly expands and connects with a broadly saccate unicellular gland located in the tissue of the chord. The somewhat hexagonal lip region is distinctly set off by a constriction, and especially laterally, on account of the large and conspicuous amphids. The very

small left ventrally submedian toothlike onchium is only 6 μ long and 1½ μ wide at the base. The second portion of the pharynx is lined throughout the greater portion of its length with a multitude of excessively fine denticles, apparently similar to those found in the pharynx of some mononchs, and, as there, slightly larger and more regular anteriorly. The entire pharynx is nearly 60 μ long. Prerectum about five times as long as the corresponding width of the body. Rectum about as long as the anal body diameter. Nothing is known concerning the sexual organs.

Found in soil by J. R. Christie at Falls Church, Va., August 29, 1922. Only one young specimen seen. Examined and measured alive in water, and afterwards fixed in Flemming's solution and examined in glycerin.

SECTONEMA

TECHNICAL DESCRIPTION

Sectonema, n. g.

Diagnosis.—Characters of Dorylaimus, with these differences: Tooth located in a ventral niche of the pharynx, grooved dorsally, and used with a slashing movement. Vestibule elongate. Guiding ring for tooth absent.

Type.—*Sectonema ventralis*, n. sp.

Sectonema ventralis, n. sp. (Fig. 18.)

Diagnosis.—Characters of the genus and others as follows:

			14.0	14.0		
1.0	3.2	16.0	52.0		99.3	6.0–10 mm.
0.3	.9	1.5	1.7		1.0	

The thick cuticle is marked by fine crisscross lines similar to those found on certain Mermithidae (7). (Fig. 18, H.) The neck tapers to the narrow lip region, which is only one-fifth as wide as the base of the neck. The blunt rounded tail is almost hemispherical in form. The two series of lateral pores are a distinct feature, especially on the tail, where they are easily seen. The first pore is located just back of the amphid, and there is but a single line until near the base of the neck, where there is a divergence into two lines, which continue to the terminus of the body. Near the head are dorsal and ventral pores. The six distinct lips are set off by a deep constriction. There are 6 papillae in the inner and 10 in the outer circlet. From a face view the vestibule and mouth opening are both found to be elongate. (Fig. 18, C, D.) The tooth is one-third to one-half as long as the width of the lip region and is set ventrally in the pharynx, reminding one somewhat of the tooth of Nygolaimus. It is grooved dorsally, and at its base the sides of the groove diverge to join the walls of the pharynx.

In cross section the pharynx is triquetrous. (Fig. 18, E.) It is composed of three sections arranged tandem. The first, thin walled and about as long as the tooth, forms the eversible portion. The second portion is about as long as the first, thickly cuticularized and forming a strong base for the attachment of the tooth. Dorsally this second portion does not appear to be differentiated from the third. The third part is three times as long as the second and narrows to join the lining of the esophagus.

When the nema attacks its prey the tooth is thrust out and the body of the victim is cut by a dorsal to ventral slash of the tooth. This slashing movement of the tooth apparently is accomplished by the use of the onchial muscle. (Fig. 18, A, *msc on.*)

The posterior three-fourths of the esophagus is enlarged by a gradual expansion. At the point where this expansion takes place there are three conspicuous pores in the lining of the esophagus, one dorsal, the other two subventral. The intestine is two-thirds as wide as the body, thick walled, and about 12 cells in circumference. It is almost always gorged with the remains of enchytraeids, which apparently form the principal food of this voracious species. Rectum as long as the anal body diameter, prerectum three to five times as long as the rectum.

Ovaries symmetrical and reflexed two-thirds their length, the front being on the left, the rear on the right side of the body. Eggs about as long as the vulva body diameter; as many as eight may occur in the body at one time.

This is one of the largest free-living nemas found in the Western States. It closely resembles *Dorylaimus regius* De Man in all respects except those of the tooth and pharynx. Frequently it is found associated with *D. regius*, which, like it, also feeds on oligochaetes (?). No males have been observed among several hundred specimens. From cultivated fields of Utah, Idaho, Colorado, and California.

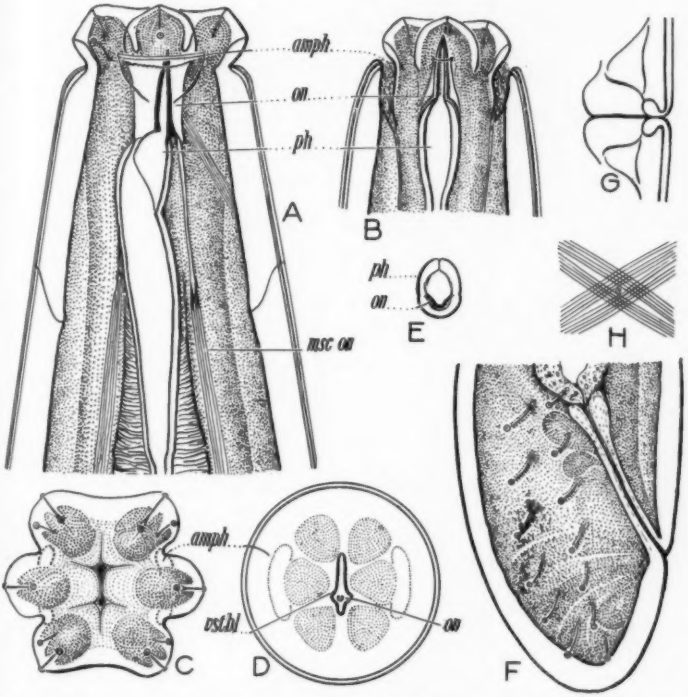

FIGURE 18.—*Sectonema ventralis*, n. g, n. sp.: A, Head, lateral view; *amph*, amphid; *msc on*, muscle of tooth; *on* tooth; *ph*, pharynx; B, lip region, dorsoventral view; C, face view; *amph*, amphid; D, cross section through pharynx near base of tooth; *amph*, amphid; *on*, tooth; *vstbl*, vestibule; E, cross section of pharynx at base of tooth; *ph*, pharyngeal wall; F, tail; G, vulvar region; H, crisscross markings of cuticle showing one of the lateral pores magnified about 2,500 times

LITERATURE CITED

(1) COBB, N. A.
1913. NEW NEMATODE GENERA FOUND INHABITING FRESH WATER AND NON-BRACKISH SOILS. Jour. Wash. Acad. Sci. 3: 432–444, illus.

(2) ———
1922. A NEW SPECIES OF NYGOLAIMUS, AN OUTSTANDING GENUS OF THE DORYLAIMIDAE. Jour. Wash. Acad. Sci. 12: 416–421, illus.

(3) FILIPJEV, I. N.
1928. NEMATODES LIBRES DU FLEUVE OKA. Okskaia Biologicheskaia Stantsiia, Murom (Arb. Biol. Oka-Sta.-Murom) 5: 81–112. [In Russian, résumé in French.]

(4) MAN, J. G. DE
1884. DIE, FREI IN DER REINEN ERDE UND IM SÜSSEN WASSER LEBENDEN NEMATODEN DER NIEDERLÄNDISCHEN FAUNA. EINE SYSTEMATISCH-FAUNISTISCHE MONOGRAPHIE. 206 p., illus. Leiden.

(5) MICOLETZKY, H.
1925. ZUR KENNTNIS TROPISCHER, FREILEBENDER NEMATODEN AUS SURINAM, TRINIDAD UND OSTAFRIKA. Zool. Anz. 64: 1–50, illus.
(6) STEINER, G.
1914. FREILEBENDEN NEMATODEN AUS DER SCHWEIZ. Arch. Hydrobiol. Planktonkunde 9: [259]–276, [420]–438, illus.
(7) ———
1924. ON SOME PLANT PARASITIC NEMAS AND RELATED FORMS. Jour. Agr. Research 28: 1059–1066, illus.
(8) THORNE, G.
1927. THE LIFE HISTORY, HABITS, AND ECONOMIC IMPORTANCE OF SOME MONONCHS. Jour. Agr. Research 34: 265–286, illus.

A PRELIMINARY REPORT OF THE RELATION BETWEEN YIELD OF WINTER WHEAT AND MOISTURE IN THE SOIL AT SEEDING TIME[1]

By A. L. HALLSTED, *Associate Agronomist, in charge of Cooperative Investigations of the Office of Dry Land Agriculture, United States Department of Agriculture, at the Fort Hays Substation of the Kansas Agricultural Experiment Station*, and E. H. COLES, *Superintendent, Colby Substation, Kansas Agricultural Experiment Station*[2]

INTRODUCTION

One of the problems that presents itself to the Kansas wheat farmer nearly every fall is whether conditions are such as to warrant seeding a large or a small acreage. Seasons in which the yields are not sufficient to return a profit, regardless of price or seed-bed preparation, are not uncommon. Obviously a reliable method of predicting yields would be of inestimable value, since acreage could be materially reduced in those years when prospects are poor and increased in others. As a matter of fact, farmers do habitually determine the acreage to be planted somewhat according to the prospects for a profitable yield based on the best available information. Thus in very dry falls or with ground in poor condition for other reasons a small acreage is likely to be seeded, whereas the opposite is true when moisture is plentiful and conditions are favorable for germination. However, the information on which such changes in acreage are made is very meager, indefinite, and often erroneous. For example, a good rain about seeding time may produce ideal topsoil conditions and thus encourage a large acreage without materially increasing the chances for a profitable yield if the subsoil is dry.

The studies on which this paper is based were undertaken to supply more accurate and dependable information regarding the relation between certain factors and the yield of the crop. It is a matter of common knowledge that moisture exerts a dominating influence on yields in western Kansas, and hence a study of factors influencing yields can not ignore this relationship. Moreover it would appear that moisture in the soil at seeding time especially merits attention (1) because of its possible relation to yield, and (2) because it can be determined accurately and without great expense.

That there is a relation between the moisture in the soil at seeding time and the yield of the following crop has been shown by Call and Hallsted.[3] The utilization of soil moisture by spring wheat and the possible value of soil moisture as a factor in forecasting yields have been discussed by Cole and Mathews.[4] The storage of water in soil and its utilization by spring wheat have been studied by Mathews,[5]

---

[1] Received for publication Apr. 28, 1930; issued October, 1930. Contribution from the Fort Hays branch of the Kansas Agricultural Experiment Station and the Office of Dry Land Agriculture, U. S. Department of Agriculture, cooperating.

[2] The authors desire to acknowledge the assistance of Prof. S. C. Salmon, of the Kansas Agricultural Experiment Station, especially in the statistical interpretation of the data.

[3] CALL, L. E., and HALLSTED, A. L. THE RELATION OF MOISTURE TO YIELD OF WINTER WHEAT IN WESTERN KANSAS. Kans. Agr. Expt. Sta. Bul. 206, 34 p., illus. 1915.

[4] COLE, J. S., and MATHEWS, O. R. USE OF WATER BY SPRING WHEAT ON THE GREAT PLAINS. U. S. Dept. Agr., Dept. Bul. 1004, 33 p., illus. 1923. (With an introduction by E. C. Chilcott.)

[5] MATHEWS, O. R. STORAGE OF WATER IN SOIL AND ITS UTILIZATION BY SPRING WHEAT. U. S. Dept. Agr. Dept. Bul. 1139, 28 p., illus. 1923. (With introduction by E. C. Chilcott.)

and recently Alsberg and Griffing [6] have emphasized the need of further work of this nature.

The data reported here were secured as a part of the investigations conducted by the Office of Dry Land Agriculture, United States Department of Agriculture, and the Kansas Agricultural Experiment Station cooperating at the Fort Hays branch station, Hays, Kans. The Fort Hays branch station is located at Hays, Ellis County, in west-central Kansas not far from the center of the Kansas hard red winter-wheat belt. The average precipitation recorded by the United States Weather Bureau for a 61-year period, 1868 to 1928, inclusive, is 22.8 inches. The average recorded by a rain gage on the project for the period during which these data were collected, namely, 1909 to 1928, inclusive, is 21.4 inches. Of this amount an average of 16.7 inches, or 78 per cent, fell during the growing season, April 1 to September 30, inclusive. The average evaporation from a free water surface during the growing season for the years 1909 to 1928, inclusive, is 46.7 inches.

The soil is a dark grayish-brown silty clay loam. The surface soil is 12 to 15 inches deep. It is a heavy soil as shown by its water relations, but its strong flocculation and granulation make the surface open and friable except when packed after heavy rains. It blows easily when the surface is smoothed by packing rains or by cultivation and after winter weathering.

The moisture-carrying capacity to a depth of 6 feet is about 22.6 per cent of the dry weight of the soil. The moisture-carrying capacity of the surface soil is slightly higher than that of the subsoil. The wilting coefficient is about 16.5 per cent. The minimum point of exhaustion for wheat as shown by observations is about 12.4 per cent.

The investigations at the Fort Hays branch station were begun in 1906 and had as their objective the study of tillage methods, rotations, and methods of conserving moisture for various crops grown in western Kansas. Soil-moisture studies in relation to wheat production have from the first taken a prominent part in these investigations, and they have been increased from time to time. The tillage and rotation project as a whole includes 461 tenth-acre plots, of which 383 relate to wheat production either directly or indirectly. More than 150 of the latter have been sampled for moisture at one time or another; about 50 individual plots comprising 483 plot years are included in the study presented here.

EXPERIMENTAL PROCEDURE

Soil samples for moisture determinations were taken at four locations on each plot, a tube cutting a core 20 mm. in diameter being used. The cores were extracted in 1-foot sections and placed in airtight metal containers. They were weighed in grams and milligrams and dried in an oven at a temperature ranging between 100° and 110° C. The moisture was calculated separately on each foot as a percentage of the dry weight of the soil.

Soil-moisture determinations have been made at various times during the period of seed-bed preparation and during the development of the crop, at all depths to and including 6 feet. Occasional deter-

[6] ALSBERG, C. L., and GRIFFING, E. P. FORECASTING WHEAT YIELDS FROM THE WEATHER. Food Research Inst., Wheat Studies v. 5, no. 1, 44 p. 1928.

minations have been made to a depth of 10 feet. Casual studies of the data revealed a closer relation between yield and moisture in the soil at seeding time than between yield and moisture at any other time of the year. For this reason and because the purpose here is to derive information of use at or preceding the sowing of the crop, attention in this paper is confined to the moisture in the soil at or near seeding time, September 15 to October 1.

The method of study adopted was to make scatter diagrams showing the relation between moisture in the soil and yield and to calculate the coefficient of correlation between these two variables. For these purposes the percentage of moisture in the soil in the first, second, and third foot sections taken together and also in the fourth, fifth, and sixth foot sections taken together were correlated with yield. In some cases the correlation between yield and the moisture

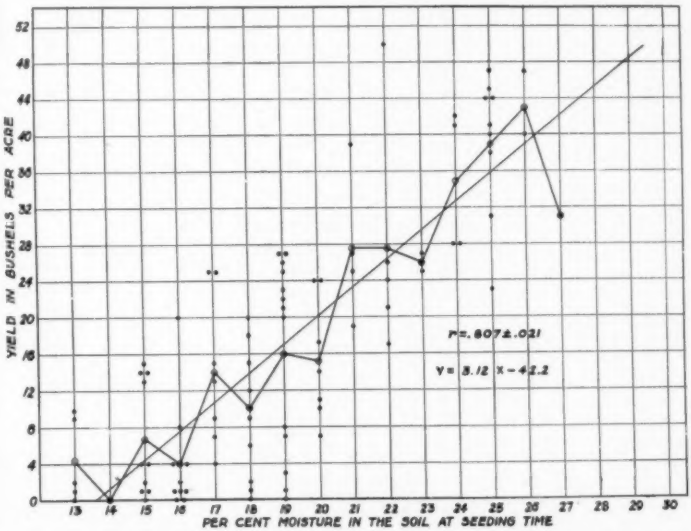

FIGURE 1.—Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following winter wheat

content of the full depth of 6 feet was calculated, but in all cases the figures so obtained were less useful for the present purpose than the others, and further consideration of these data is reserved for future study. The moisture determinations are reported to tenths of a per cent and the yields to tenths of a bushel. In calculating the correlation coefficients the decimals in both cases were disregarded, five-tenths and above being carried to the next whole number and decimals less than five-tenths being dropped.

Correlations were first calculated for single plots or for small groups of plots having identical or nearly identical treatment, and these were then grouped together where further study indicated that such grouping was justified. This might have been carried even further by combining some of these larger groups, but the data are not sufficient to indicate clearly in just what cases this might be done, and hence this possibility is reserved for future study.

EXPERIMENTAL DATA

Table 1 shows the previous crop and the treatment, the period of years covered, number of plot years, mean yield, mean moisture content of the first and the second 3-foot layers of soil, standard deviations of moisture and of yields, and the correlation between the moisture and the yield in each of the two soil layers. Table 2 gives the same information for larger groups, the basic data being the same in every case except for wheat following corn, in which case Table 2 contains some data that are not given in Table 1. Figures 1 to 6 are scatter diagrams showing the relations between the percentage of moisture in the surface 3 feet of soil at seeding time and the yields of wheat grown in the different cropping systems. Regression lines are shown in all cases except Figures 3 and 4, in which cases regression probably is not linear.

TABLE 1.—*Moisture content at seeding time, yield of wheat, standard deviations, and coefficients of correlation between soil-moisture content and yield of wheat grown in several cropping systems at the Fort Hays branch station, Hays, Kans.*

Group	Previous crop	Plot or rotation No.[a]	Period covered	Plot years	Mean yield per acre	Standard deviation of yield	Mean moisture in—		Standard deviation of moisture in—		Correlation coefficient for—	
							First, second, and third foot layers	Fourth, fifth, and sixth foot layers	First, second, and third foot layers	Fourth, fifth, and sixth foot layers	First, second, and third foot layers	Fourth, fifth, and sixth foot layers
				No.	*Bush.*	*Bush.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		
1	Wheat	A	1910–1928	17	9.5	9.7	16.6	14.5	3.8	1.3	0.862±0.042	0.308±0.148
	----do	B	1910–1928	17	16.5	12.4	19.5	15.4	3.4	1.1	.800± .059	.418± .135
	----do	E	1911–1928	16	18.8	13.9	19.9	15.6	3.4	1.4	.807± .059	.485± .104
	----do	F	1911–1928	16	18.7	12.5	20.0	15.8	3.3	1.3	.825± .054	.666± .094
	----do	401	1917–1928	9	21.8	17.1	19.8	[b] 17.8	2.4	[b] 1.4	.742± .101	[b] .412± .198
	----do	403	1917–1928	8	22.4	14.5	19.8	17.9	3.6	1.9	.847± .067	.148± .233
	----do	404	1917–1928	9	19.7	15.2	19.0	16.8	3.3	2.3	.900± .043	−.095± .223
2	Barley	403	1918–1928	8	25.4	15.0	20.9	18.3	3.0	2.1	.778± .094	.207± .228
	----do	404	1917–1928	9	21.9	15.2	20.3	17.0	3.0	2.0	.906± .040	−.313± .205
3	Corn	53	1911–1927	13	12.8	10.5	18.7	17.3	2.2	1.6	.484±4.126	.768± .080
	----do	54	1911–1927	13	14.9	12.2	18.5	18.5	2.1	2.2	.692± .098	.220± .105
4	Kafir	349	1916–1928	11	11.5	9.2	15.4	[c] 17.1	1.9	[c] 1.3	.326± .182	[c] −.495± .154
	----do	350	1916–1928	11	15.3	11.8	17.1	[c] 18.1	2.3	[c] 1.7	.347± .179	[c] .194± .216
5	Rye for green manure.	51	1914–1921	7	17.4	9.1	20.4	16.6	2.2	1.6	.763± .106	−.174± .247
	----do	55	1911–1927	15	15.8	10.0	20.3	[d] 17.2	2.3	[d] 2.3	.568± .118	[d] .537± .133
	Peas for green manure.	56	1911–1927	15	16.6	11.6	20.1	[d] 18.2	3.0	[d] 3.2	.648± .101	[d] .126± .184
	----do	92	1914–1921	7	18.7	10.2	19.6	16.9	2.6	1.6	.485± .195	−.470± .255
6	Fallow.											
	[b]	CC C–D	1910–1928	17	26.4	13.8	24.0	[e] 19.8	1.6	[e] 2.1	.442± .135	[e] .065± .168
	[b]	MF H to S	1915–1922	46	24.2	12.7	22.9	20.4	2.4	3.0	.416± .084	.419± .082
	[b]	57, 401, 402	1913–1928	30	22.7	12.4	23.4	19.0	2.3	2.3	.562± .084	.360± .107
	[b]	501 to 510, 551 to 560.	1914–1921	160	23.5	12.5	22.9	19.7	2.4	2.7	.471± .042	.413± .044

[a] The cropping systems indicated in the third column are as follows: Group 1. Plots A, B, E, and F are continuously cropped to wheat; plot A is late fall plowed; B is early fall plowed; E is early fall plowed and subsoiled; and F is early fall listed. Plots 401, 403, and 404 are 4-year rotations including various crops. In these plots wheat follows wheat. Group 2: Nos. 403 and 404 are 4-year rotations in which wheat follows barley. Group 3: Nos. 53 and 54 are 4-year rotations in which wheat follows corn. Group 4: Nos. 349 and 350 are 2-year rotations in which wheat follows kafir. Group 5: Nos. 51 and 55 are 4-year rotations in which wheat follows rye turned under for green manure and 56 and 92 are 4-year rotations in which wheat follows Canada field peas turned under for green manure. Group 6: Plots CC C–D and MF H to S inclusive, are plots alternately cropped and fallowed. Nos. 57, 401, and 402 are 4-year rotations in which wheat follows fallow. Nos. 501 to 510, inclusive, and 551 to 560, inclusive, are 3-year rotations in which wheat follows fallow.

[b] 8 plot years. [c] 9 plot years. [d] 13 plot years. [e] 16 plot years. [f] See Group 6, footnote *a*.

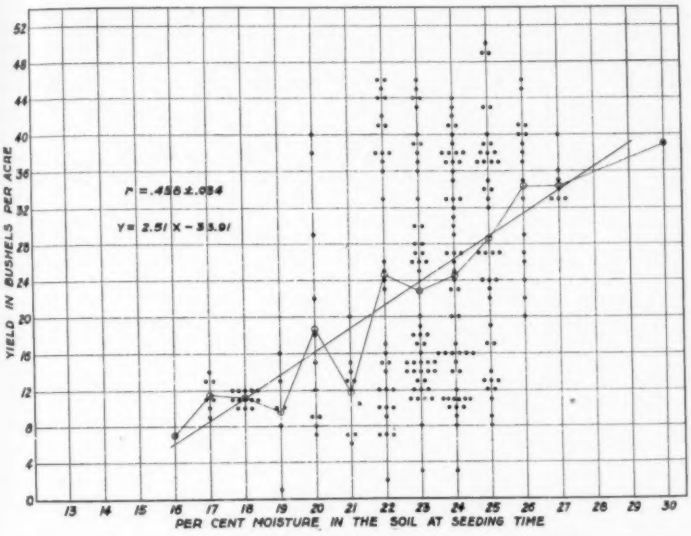

FIGURE 2.—Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following fallow

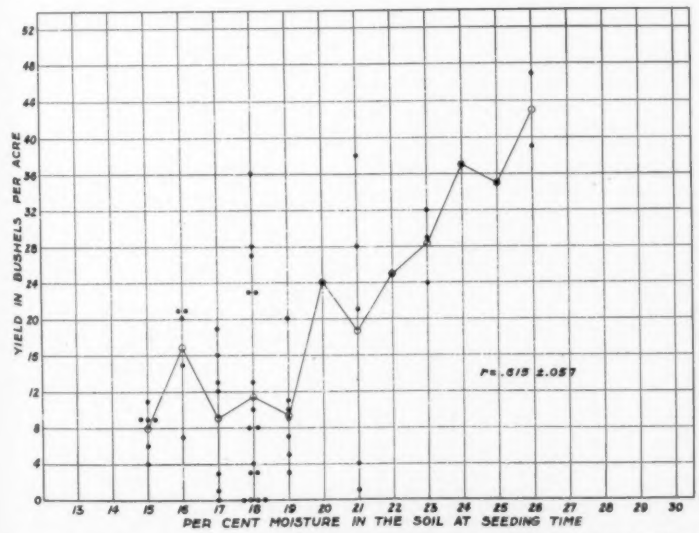

FIGURE 3.—Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following corn

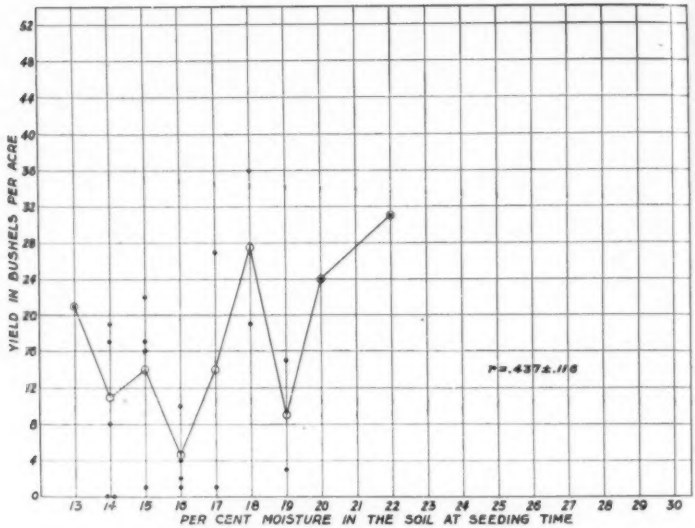

FIGURE 4.—Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following kafir

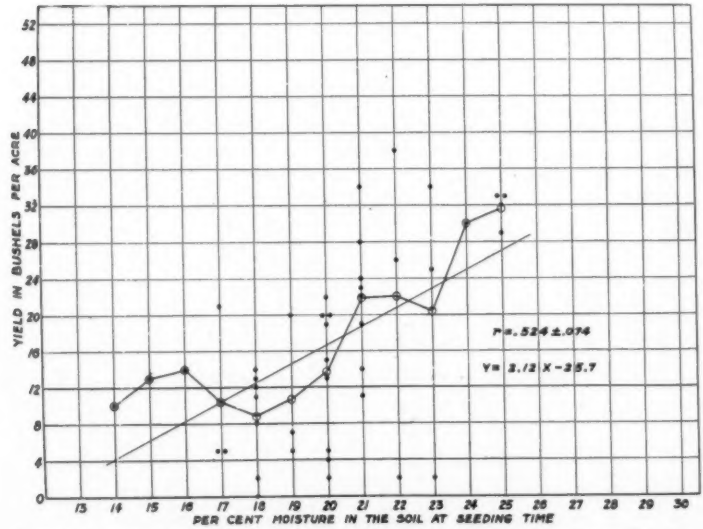

FIGURE 5.—Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following green manure

For all groups in Table 2, the correlations between moisture content of the first or surface 3 feet of soil and yield of wheat are significant in relation to the probable errors, showing an unmistakable relation between the two. There is likewise a significant correlation between yield and moisture in the second 3 feet for wheat after wheat, wheat after corn, and for wheat after fallow. In other cases the correlation is practically zero. In every instance but one the correlation with the moisture in the first 3 feet of soil is significantly higher than with the moisture in the second 3 feet. In this one exception, which was after fallow, the correlation for each section is relatively low.

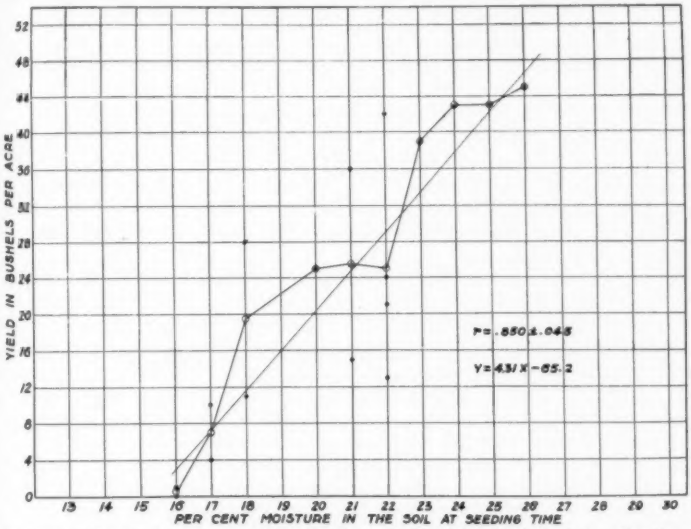

FIGURE 6.—Relation between percentage of moisture in the surface 3 feet of soil at seeding time and the yield of winter wheat following barley

TABLE 2.—*The relation between moisture content in the soil at seeding time and yield of wheat grown in various cropping systems at the Fort Hays branch station*

[Results arranged in larger groups]

Previous crop	Period covered	Plot years	Mean yield per acre	Standard deviation of yield	Mean moisture in—		Standard deviation of moisture for—		Correlation coefficient for—	
					First, second, and third foot layers	Fourth, fifth, and sixth foot layers	First, second, and third foot layers	Fourth, fifth, and sixth foot layers	First, second, and third foot layers	Fourth, fifth, and sixth foot layers
		Number	*Bush.*	*Bush.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		
Wheat	1910–1928	92	17.3	13.9	19.1	[a] 15.9	3.6	[a] 1.8	0.807±0.021	[a] 0.402±0.059
Barley	1917–1928	17	23.5	15.2	20.6	17.6	3.0	2.1	.850±.045	.027±.164
Corn	1911–1928	55	15.1	12.1	18.7	[b] 17.8	2.8	[b] 2.3	.615±.057	.342±.085
Kafir	1916–1928	22	13.4	10.7	16.2	[c] 17.6	1.7	[c] 1.6	.437±.110	[c] .012±.144
Green manure	1911–1927	44	16.8	10.5	20.1	[d] 17.4	2.6	[d] 2.5	.524±.074	[d] .145±.105
Fallow	1910–1928	253	23.8	12.6	23.0	[e] 19.7	2.3	[e] 2.7	.458±.034	[d] .403±.036

[a] 91 plot years. [b] 49 plot years. [c] 18 plot years. [d] 40 plot years. [e] 252 plot years.

A complete and critical study of the relation of soil moisture to yield should take into consideration the supply of moisture in the lower depths. Thus, it is conceivable that a correlation of 0.402 as was secured for the yield and moisture in the second 3-foot layer on wheat after wheat may be useful when considered in connection with the correlation in the upper 3 feet. The same applies to wheat on fallow and to wheat on corn ground. Some consideration has been given to these possibilities, but a preliminary study by means of multiple and partial correlations indicated that so far as the purpose of the present paper is concerned, no great improvements would be secured by the use of these data. The present report, therefore, is based on the first 3 feet of soil and the matter of the lower depths is reserved for future study. For the practical purpose of predicting yields, it is fortunate that the correlation between yield and moisture in the first 3 feet is relatively high, since the determinations for only 3 feet are much easier and more convenient to make.

The highest correlations were secured where wheat was grown after wheat or barley, the coefficients being 0.807 ± 0.021 and 0.850 ± 0.045, respectively. The difference between them is less than the probable error of difference. Because of this fact and because of the small numbers involved in the latter case, no distinction between the two is made at this time.

The correlations for wheat after corn and after kafir are 0.615 ± 0.057 and 0.437 ± 0.116, respectively. These lower values are in line with expectations in indicating that factors other than total moisture in the upper 3 feet of soil play an important part in determining the yield. A dry surface soil at seeding time may result in poor germination and a thin stand. A thin stand is usually accompanied by weed growth in the spring and late maturity, which subjects the crop to a longer period of danger of damage from hot winds, rust, and insects. Corn and kafir stubble lands are also more subject to soil blowing, which sometimes reduces yields. The well-known deleterious effect of sorghums on succeeding crops may also be a factor in reducing the yield of wheat after kafir. It should be noted, however, as previously mentioned and as illustrated in Figures 3 and 4, regression is probably not strictly linear in these cases, and hence the relation between moisture and yield may be closer than the calculated values of r suggest. In fact, it appears more than likely that the relation between soil moisture and yield when the moisture content of the soil is less than about 20 per cent is different than when it is 20 per cent or more. However, the available data are hardly sufficient for a critical discussion of these relationships.

The correlations where wheat is grown after green manure (0.524 ± 0.074) and after summer fallow (0.458 ± 0.034) are nearly the same, the difference being less than the probable error of difference. Wheat grown on land prepared by either of these methods sometimes suffers from the same causes, and the explanation for the relatively low correlation is probably the same for each. During the period here considered, there have been years when the rainfall was not enough or was too poorly distributed to finish properly the heavy growth produced on fallowed or green-manured plots. When such conditions prevail until after the stored moisture becomes exhausted, what promised to be a heavy yield may suddenly become an almost complete failure. Such conditions obtained in 1911, 1913, 1917, and 1927.

On the other hand, in seasons of high rainfall, such wheat makes a heavy growth and is more subject to injury from lodging. Under these conditions it often yields even less than cropped land. This was the case in 1915 and 1919.

A correlation of 0.8 or more, as was secured for wheat after wheat and wheat after barley, should be of value in predicting yields on the basis of moisture in the soil at seeding time. Consideration of the graphs (figs. 1 to 6) also will permit certain useful predictions regarding yield even when the correlation is considerably less than 0.8. For purposes of prediction the regression equation is useful, and consequently it has been calculated for each set of data and is shown in the figures. In two cases (to be noted later) the regression is not strictly linear. The same is perhaps true in other cases, although, in general, the relation is so nearly linear as to introduce no material error in considering it so. The numbers involved in general are too small to justify calculation of the correlation ratio.

Figure 1 shows a total of 92 comparisons of the relation between the water content at seeding time of land that has borne a crop of wheat and the yield of the following crop of wheat. In 35 of these the water content of the surface 3 feet averaged 20 per cent or more, which by the regression formula would indicate a probable yield of 20 bushels or more per acre. The yield was less than the indicated minimum in only 7 of the 35 cases. In only 1 of these 7 cases could the yield be characterized as very poor.

In 49 of the 92 comparisons the soil at seeding time contained 18.5 per cent of water or more, which would indicate a yield of 15 bushels or more. In only 9 of these 49 cases was the yield less than 15 bushels. In only 6 cases was the yield less than 10 bushels per acre.

TABLE 3.—*Calculated and actual wheat yields when three different methods were used in preparing wheat stubble land for planting*

[$y=3.12x-42.24$]

Crop year	Late fall plowed			Early fall plowed			Early fall listed		
	Soil moisture	Yield per acre		Soil moisture	Yield per acre		Soil moisture	Yield per acre	
		Calculated	Actual		Calculated	Actual		Calculated	Actual
	Per cent	*Bushels*	*Bushels*	*Per cent*	*Bushels*	*Bushels*	*Per cent*	*Bushels*	*Bushels*
1910	22	26.4	20.5	24	32.6	27.8			
1911	14	1.4	0	19	17.0	.3	18	13.9	0.6
1912	13	0	2.3	15	4.6	13.8	19	17.0	26.6
1913	16	7.7	.8	16	7.7	2.3	16	7.7	8.4
1914	19	17.0	20.6	21	24.3	24.8	19	17.0	23.1
1915	13	0	9.3	15	4.6	13.1	15	4.6	13.9
1916	18	13.9	8.7	25	35.8	22.7	23	29.5	23.1
1917	13	0	0	18	13.9	2.0	16	7.7	0
1918	13	0	9.7	17	10.8	14.9	23	29.5	24.8
1919	(a)								
1920	19	17.0	19.9	20	20.2	24.4	22	26.4	24.2
1921	15	4.6	4.2	21	24.3	26.9	23	29.5	27.1
1922	13	0	.5	17	10.8	6.8	17	10.8	13.3
1923	(b)								
1924	27	42.0	31.1	25	35.8	38.1	26	38.9	40.3
1925	16	7.7	0	16	7.7	1.2	17	10.8	3.7
1926	16	7.7	3.5	20	20.2	14.8	21	24.3	19.2
1927	15	4.6	3.5	18	13.9	6.3	20	20.2	6.8
1928	20	20.2	24.2	25	35.8	41.2	25	35.8	44.2

[a] No moisture determinations near seeding time were made of 1919 crop.
[b] Crop of 1923 was destroyed by hail.

On the other hand, there are 26 cases where the moisture in the soil was 16.7 per cent or less, indicating a yield not exceeding 10 bushels. In only 6 cases was a yield of as much as 10 bushels obtained, and in only 1 of the 6 was the yield as much as 20 bushels per acre. In 18 of the 26 cases the yield was less than 5 bushels per acre.

It may be of interest to pursue this comparison somewhat further, and for this purpose Table 3 has been prepared. This table gives the moisture at seeding time in soils prepared by three different methods, the yield that would have been predicted by the regression equation, and the actual yield.

The correlation for moisture and yield on fallow was found to be only 0.458, hence any predictions of yield are certain to be less accurate than for wheat after wheat. Nevertheless, it appears that the relation is sufficiently close to be of value. Figure 2 shows that there were 226 cases out of a total of 253 in which there was 20 per cent of moisture in the soil at seeding time. According to the regression equation, a yield of 15 bushels or more should have been obtained. Actually this quantity (or more) was obtained in 165 cases, or in all but 60. Of these 60 cases, only 3 may be considered as complete failures (2 to 3 bushels per acre). Less than 15 bushels per acre would have been predicted in 27 cases. In only 1 of these cases was a yield of 15 bushels or more produced.

The moisture content of the soil at seeding time, the yields that would have been predicted from the regression equation, and the actual yields for three different plots of wheat grown on fallow are given in Table 4.

TABLE 4.—*Calculated and actual yields of wheat on fallow*

[$y = 2.51\ x - 33.91$]

Crop year	Soil moisture	Yield per acre		Soil moisture	Yield per acre		Soil moisture	Yield per acre	
		Calculated	Actual		Calculated	Actual		Calculated	Actual
	Per cent	*Bushels*	*Bushels*	*Per cent*	*Bushels*	*Bushels*	*Per cent*	*Bushels*	*Bushels*
1910	25	28.8	42.5	[a]			[a]		
1911	24	26.3	2.6	[a]			[a]		
1912	20	16.3	29.2	[a]			[a]		
1913	22	21.3	10.3	24	26.3	16.4	[a]		
1914	25	31.4	21.6	24	26.3	19.9	25	28.8	26.8
1915	23	23.8	11.3	23	23.8	17.5	23	23.8	12.5
1916	27	33.9	33.8	25	28.8	31.8	26	31.4	37.8
1917	22	21.3	7.1	24	26.3	7.9	25	28.8	8.8
1918	23	23.8	35.8	18	11.3	9.8	19	13.8	7.9
1919	[a]			23	23.8	15.3	23	23.8	16.3
1920	25	28.8	37.0	27	33.9	33.0	25	28.8	26.7
1921	24	26.3	37.1	24	26.3	42.3	25	28.8	39.6
1922	24	26.3	25.2	23	23.8	32.5	24	26.3	24.3
1923	[b]								
1924	26	31.4	45.6	[a]			[a]		
1925	21	26.3	23.3	21	26.3	22.3	25	28.8	22.2
1926	24	26.3	26.2	21	18.8	19.7	21	18.8	12.8
1927	24	26.3	10.8	24	26.3	16.0	22	21	10.3
1928	25	28.8	48.7	25	28.8	49.0	25	28.8	50.0

[a] No moisture determinations were made near enough to seeding time to be of value.
[b] The 1923 crop was destroyed by hail.

Although the correlations are not high, 0.615 and 0.437, 20 per cent moisture in the upper 3 feet of soil on corn or kafir land has nearly always produced a crop, only 2 failures out of a total of 14 cases having been recorded. However, it is more difficult to store

as much as 20 per cent of moisture in the soil after these crops than after small-grain crops because corn and kafir mature several weeks later than the small grains and therefore afford a much shorter time in which to accumulate moisture before seeding time for wheat. Thus, of the 55 cases in which wheat has been grown after corn, there were only 14, or slightly more than 25 per cent, in which the moisture content of the upper 3 feet of soil was 20 per cent or more. Of the 22 cases of wheat after kafir (fig. 4), only 2 had 20 per cent or more.

With less than 20 per cent in the upper 3 feet of soil, the average yields are low and uncertain. Thus, for this group the average yield after corn is 10.9 bushels and on kafir ground 12 bushels.

DISCUSSION AND CONCLUSIONS

With reference to the practical side of the problem, it has already been pointed out that a moisture content of 20 per cent or more in the upper 3 feet of soil at seeding time at Hays has practically precluded a crop failure as a result of drought, although failure may result from hail, flood, or other conditions. This fact should be of considerable practical value, not only in discouraging a large acreage when the supply of moisture is materially less than 20 per cent, but also by way of emphasizing the need for those practices which will conserve the most moisture in the soil. In the period covered by these studies it has been possible by means of fallow to conserve as much as 20 per cent of moisture in the upper 3 feet of soil in nearly 90 per cent of the cases, for wheat after small grains in about 40 per cent of the cases, for wheat after corn in about 25 per cent of the cases, and for wheat after kafir in only about 10 per cent of the cases.

With a soil-moisture content of much less than 20 per cent in the surface 3 feet at seeding time the chances of securing a large crop are much less and the chances for harvesting a small crop or having a failure are measurably increased. With about 15 per cent moisture in the soil, the crop is practically dependent on rainfall subsequent to seeding.

While within certain limits it appears that the data presented here may be of considerable value for forecasting yields, they are principally useful in predicting failures, or more accurately, as a means of determining when chances for a crop are reasonably good and when they are largely dependent on factors other than stored moisture. The smaller the quantity of stored moisture at seeding time, the more dependent is the crop on the weather during the growing season and the greater are the chances of a failure.

COLOR OF POTATO CHIPS AS INFLUENCED BY STORAGE TEMPERATURES OF THE TUBERS AND OTHER FACTORS[1]

By MARION DEYOE SWEETMAN

Associate in Home Economics Research, Maine Agricultural Experiment Station

INTRODUCTION

In the fall of 1928 an inquiry was received at the Maine Agricultural Experiment Station from a manufacturer of potato chips in regard to the cause of dark color[2] in potato chips. He stated that frequently in the winter he had difficulty with northern-grown potatoes, because potato chips made from them were dark colored. A later survey showed that this has been the experience of other chip producers to the extent that some discriminate definitely against northern-grown potatoes. Furthermore, wholesale receivers and jobbers on the Boston market have reported that they have occasional complaints from proprietors of hotels and restaurants about the color of French-fried potatoes made from certain lots of these tubers.

Vosbury (*9*)[3] made a study of some phases of potato-chip making and concluded that the troubles of manufacturers are generally due to the use of varieties not adapted to chip making, to improper washing of the sliced potatoes, or to the use of oil at too low temperatures for frying. She did not mention any outstanding differences in color. Woodruff and Blunt (*10*) state that they found that browning of chips varied in different fats, but their data do not give any clues as to the reasons for the darkening that commercial producers find associated with certain lots fried under their usually standardized conditions.

It was known that northern-grown potatoes would frequently be held in cool storage and that this increased their sugar content (*1*, *4*). Since many foods brown during cooking as a result of the caramelization of the sugar which they contain, it was suspected that it was the accumulated sugar in the potatoes which caused chips made from them to be excessively dark.

PLAN OF EXPERIMENT

In some preliminary experiments, tubers stored in a refrigerator at 35° to 40° F. fried darker than those from the same lot held at room temperature. Consequently, a series of experiments was planned for the season of 1929 to ascertain the effect of cold storage on color of chips from various lots of potatoes. Since Appleman (*1*) and Hopkins (*4*) had also reported that sugar which had accumulated in the cold disappeared when the tubers were held at temperatures above 40° F., some of each lot were to be returned to warm storage for varying periods before frying.

[1] Received for publication May 23, 1930; issued October, 1930.

[2] The word "dark" is used in this paper to refer to varying shades of brown which are darker values of the light brown or golden brown tints associated with desirable color in potato chips.

[3] Reference is made by number (italic) to Literature Cited, p. 490.

SELECTION OF POTATOES

The potatoes used were grown on the experimental farms of the Maine Agricultural Experiment Station, that is, Aroostook farm at Presque Isle in the northeastern part of the State, and Highmoor farm at Monmouth in the south-central part.

The lots of tubers were divided into two series, series A, consisting of tubers from plots in which there were no recognized signs of disease or in which diseased plants were rogued out early in the season, and series B, consisting of potatoes from plants with known disease and from rows grown next to such diseased stock so that there was strong likelihood of infection.

Series A included: (1) Green Mountain potatoes from both farms and Bliss Triumph and Irish Cobbler tubers from Aroostook Farm; (2) a set of Green Mountain potatoes dug at different dates during the growing season to provide material for study of the effects of differences in maturity at digging; (3) a collection of Green Mountain hills bagged separately at digging; and (4) potatoes from two tuber-line strains of Green Mountain. The last three collections were grown at Highmoor farm.

Series B included Green Mountain tubers produced at both farms by plants showing, or by healthy plants near those showing, the following diseases: Spindle tuber, leaf roll, rugose mosaic, mild mosaic, and giant hill. Symptoms of these diseases as they appear in Maine have been described by Schultz (*6*) and by Schultz and Folsom (*7*).

All Green Mountain tubers were from plots supervised and rogued by Donald Folsom, plant pathologist at the Maine Agricultural Experiment Station.

CONDITIONS OF STORAGE

With the exception of those potatoes in series A dug at earlier dates to show the effects of immaturity, all were harvested late in September or early in October and shipped immediately to Orono. Here they were stored in a vegetable cellar where thermograph records showed that the temperature seldom went below 40° F., and then for only a few hours at a time. The upper limit of the range was around 55° F.

On October 18 eight samples of each of the above kind of potatoes were selected and placed separately in paper bags. These were regrouped to form eight lots which were handled as indicated later. The cold storage was commercial and was said to vary from 32 to 37° F. Warm storage was in rooms which varied from 60 to 70° F.

The experimental lots were held under the conditions outlined below:

Lot 1.—Fried immediately as control.
Lot 2.—Fried after two weeks in cold storage.
Lot 3.—Control, held in cellar and fried two weeks after lot 1.
Lot 4.—Fried after four weeks in cold storage.
Lot 5.—Fried after four weeks of cold storage followed by one week of warm storage.
Lot 6.—Fried after four weeks of cold storage followed by two weeks of warm storage.
Lot 7.—Control, held in cellar and fried seven weeks after lot 1.
Lot 8.—In cold storage four weeks. Then each sample of 10 tubers was divided, and one-half was fried after warm storage for four weeks and the remainder after warm storage for eight weeks.

EXPERIMENTAL METHODS

FRYING TECHNIC

It was of course necessary to standardize the frying technic to eliminate variations due to differences in temperature and in time of cooking. Unless otherwise stated, 10 tubers were taken as a sample. These were weighed and then measured with a sliding caliper so that it might be possible to correlate any differences with size and shape of the tuber.

Each tuber was divided transversely, approximately in the middle. One-half was thinly pared to about 1 centimeter back from the cut surface and two slices one-sixteenth of an inch (0.16 centimeters) in thickness were cut from the median surface with a vegetable slicer for frying. Tests showed that these represented the tuber adequately. All of the chips from one sample (10 potatoes) were washed in running water for the few minutes before the next sample was ready. Then they stood in pans of cold water until cooked. This period was usually about an hour.[4] The slices were blotted dry with paper towels before being placed in the hot oil.

For the frying, a uniform amount (1,000 gm.) of a high-grade cottonseed oil was heated in a heavy, straight-sided aluminum pan at constant rate of heating on an electric hot plate. The pan was of such size that the oil was 6 centimeters deep. Ten chips, one from each potato in the sample, were dropped into the oil when the thermometer indicated a temperature of 400° F. They cooled the oil, sometimes to a temperature as low as 350° in the case of the larger chips, but the continuous heating brought the temperature quickly up to about 375°, and for most of the cooking period the range was from 375 to 400°. Tests showed that frying temperatures below 350° were required to alter the color of the chips produced.

At first a stop watch was used to time the frying period, but it was found that the time required to produce chips which were properly cooked depended too much upon the cooling action of the slices (which varied with their size) for exact comparisons. However, it was easy to fix as a definite end point the instant when the chips became opaque and bubbling ceased almost completely. This cessation of bubbling is associated with the almost complete removal of the water content of the slices. The chips were kept submerged in oil by the use of a wire whip. When done, they were removed by means of the same utensil and allowed to drain on paper towels until records of color were taken.

When one sample of 10 chips was cooked it was immediately followed by the set of duplicates. The similarity of the members of each pair representing one tuber proved that the whole cooking process was sufficiently controlled to give uniform and comparable results.

COLOR SCALE

When the first controls, lot 1, were fried, five chips were selected to cover the range of colors represented. These were numbered in order beginning with the lightest. When lot 2, which had been held in cold storage two weeks, was fried the color range was extended in the direction of darkness, and two more chips were added to the scale.

[4] Tests showed that this variation in time of soaking did not alter the frying color.

Finally an eighth very dark chip was added when lot 4, which had been in cold storage four weeks, was sampled.

It should be emphasized that the purpose was merely to get some standard for comparing color differences. Because of the method of selection of these chips, it was impossible to be certain that they represented uniformly spaced degrees of change.

To standardize this scale the method of color measurement described by Nickerson (*5*) was employed. This gave color qualities according to the Munsell notation.[5]

TABLE 1.—*Color qualities of standard potato chips according to Munsell notation* [a]

Chip No.	Hue	Brilliance	Chroma	Chip No.	Hue	Brilliance	Chroma
1	25.00	7.91	5.85	5	17.85	5.53	5.83
2	23.58	7.59	6.76	6	16.16	4.43	4.04
3	21.29	7.05	7.08	7	15.92	4.31	3.79
4	19.07	6.11	6.90	8	15.00	3.42	2.08

[a] Hue: Redness, yellowness, etc., or the quality that differentiates a color from gray of the same brilliance.
Brilliance: Lightness or darkness of a color.
Chroma: Grayness of a color.
In this notation all hues are comprehended in a series of 100 units representing subdivisions of the 10 primary and secondary hues. Thus standard red may be designated as 5, standard yellow-red as 15, standard yellow as 25, etc., and all possible hues may be represented by some whole number or decimal between 0 and 100.
The brilliance scale begins at 0, absolute black, and runs through middle gray to absolute white at 10.
The notation for chroma begins at neutral gray, 0, and progresses by equal steps to the strongest possible saturation which varies with brilliance and hue.
Complete notation is written: Hue $\frac{\text{brilliance}}{\text{chroma}}$.
In these readings the following standard Munsell disks were used: Yellow 8/9, yellow-red 6/8, neutral 9/, and neutral 1/.

The same color scale was used throughout the experiments. Each chip fried was compared with it and given the number that which it most nearly resembled. When the ratings for the 10 sets of duplicates were averaged, the result was used to represent the sample in the data that follow.

EXPERIMENTAL DATA

RELATION BETWEEN EXPERIMENTAL STORAGE CONDITIONS, CHIP COLOR, AND OTHER FACTORS

VARIETY AND CHIP COLOR

Six-ounce tubers from the Bliss Triumph, Irish Cobbler, and Green Mountain varieties grown on the two farms were compared in color of chips produced when they were held under the various storage conditions. The results are given in Table 2 as the average rating when compared with the color scale already described.

[5] A "tuber line," as the term is used here, is a seed stock, perpetuated from year to year, that is known to have originated from a single tuber.

TABLE 2.—*Color of potato chips made from tubers of different varieties held under different storage conditions*

Variety	Lot 1, control	Lot 2, stored cold 2 weeks	Lot 3, control	Lot 4, stored cold 4 weeks	Lot 5, stored cold 4 weeks and warm 1 week	Lot 6, stored cold 4 weeks and warm 2 weeks	Lot 7, control	Lot 8, first stored cold 4 weeks, then—	
								One-half stored warm 4 weeks	One-half stored warm 8 weeks
Green Mountain (H) [a]	[b] 1.6	5.0	1.9	6.6	5.4	4.9	1.9	3.4	1.6
Green Mountain (A) [b]	4.1	6.7	4.5	8.0	6.3	5.6	4.0	4.4	3.4
Bliss Triumph (A)	3.6	6.8	4.4	8.0	7.0	6.8	4.0	6.0	5.0
Irish Cobbler (A)	2.9	6.1	2.9	7.0	6.2	6.0	3.0	4.8	3.0

[a] (H) signifies that the potatoes were grown at Highmoor farm; (A) that they were grown at Aroostook farm.

[b] For meaning of numerals see discussion of color scale (p. 482). The larger the number, the darker the color.

Chips made from potatoes from Aroostook farm were conspicuously darker than those from the tubers grown at Highmoor farm. Possible reasons for this will be discussed later. In all cases cold storage increased the darkness of the chips, while succeeding warm storage, if sufficiently prolonged, resulted in what was essentially a return to the original condition. Bliss Triumph tubers showed retardation in this reversion. The controls were very nearly constant throughout the experiment.

The probable errors of the means given in the tables were not computed because the numbering of the chips in the scale was a purely arbitrary method of characterizing differences not known to be uniform. In most cases variation within the samples was so limited that only two or three scale values were represented. The trends indicated by the means are unmistakable because their differences are so large.

SIZE OF TUBER AND CHIP COLOR

Four-ounce and 8-ounce tubers of the Green Mountain variety grown on both farms were selected for comparison with the 6-ounce sizes fried in the variety tests. These results are given in Table 3.

TABLE 3.—*Color of potato chips made from tubers (Green Mountain) of different sizes held under different storage conditions*

Size (ounces)	Lot 1, control	Lot 2, stored cold 2 weeks	Lot 3, control	Lot 4, stored cold 4 weeks	Lot 5, stored cold 4 weeks and warm 1 week	Lot 6, stored cold 4 weeks and warm 2 weeks	Lot 7, control	Lot 8, first stored cold 4 weeks, then—	
								One-half stored warm 4 weeks	One-half stored warm 8 weeks
4 (A)	[a] 4.1	7.0	4.1	7.9	6.8	5.7	4.3	4.8	3.2
6 (A)	4.1	6.7	4.5	8.0	6.3	5.6	4.0	4.4	3.4
8 (A)	3.9	6.0	4.2	7.8	6.3	5.6	4.3	----	3.0
4 (H)	1.8	4.9	2.2	6.4	5.1	4.9	2.0	4.2	2.2
6 (H)	1.6	5.0	1.9	6.6	5.4	4.6	1.9	3.4	1.6
8 (H)	1.3	5.1	1.5	6.3	5.6	5.4	1.6	4.8	1.4

[a] For meaning of numerals see discussion of color scale (p. 482). The larger the number, the darker the color.

There are no significant differences between the tubers of these sizes in color of chip after different conditions of storage.

Time of Digging and Chip Color

A series of Green Mountain potatoes grown at Highmoor farm was prepared to test the effects of varying maturity by digging at intervals during the season. The digging dates were August 6, 17, and 28 and September 16 and 26. The results are shown in Table 4.

Table 4.—*Color of potato chips made from tubers (Green Mountain) dug at different dates and held under different storage conditions*

Digging date	Lot 1, control	Lot 2, stored cold 2 weeks	Lot 3, control	Lot 4, stored cold 4 weeks	Lot 5, stored cold 4 weeks and warm 1 week	Lot 6, stored cold 4 weeks and warm 2 weeks	Lot 7, control	Lot 8, first stored cold 4 weeks, then—	
								One-half stored warm 4 weeks	One-half stored warm 8 weeks
Aug. 6	[a] 2.6	6.0	3.7	7.2	6.3	6.4	3.0	6.0	4.4
Aug. 17	3.4	5.5	3.3	7.0	6.5	5.9	3.2	6.0	4.2
Aug. 28	2.5	5.5	2.5	6.4	5.9	5.5	3.1	4.6	3.2
Sept. 16	1.8	5.8	1.9	7.6	6.3	5.4	2.1	4.8	3.2
Sept. 26	1.6	5.0	1.9	6.6	5.4	4.9	1.9	3.4	1.6

[a] For meaning of numerals see discussion of color scale (p. 482). The larger the number, the darker the color.

If potatoes are dug in an immature state they produce darker chips than do the more mature, even when they are held under similar storage conditions for a few weeks before frying. There is some evidence from these data that immature potatoes which have been in cold storage respond less slowly to warmth in returning to the original condition after a preliminary period of cold storage. Chips from immature potatoes are uneven in color, as are those which have been in cold, followed by warm storage.

Genetic Similarity and Chip Color

Two tuber-line [6] stocks were studied to see whether the tubers from them would exhibit uniformity in their response to changes in environment. The data for the individual tubers are shown in Table 5.

[6] See footnote 5.

TABLE 5.—*Storage effects on the color of potato chips made from tubers of two different genetic lines*

LINE 1

	Lot 1, control	Lot 2, stored cold 2 weeks	Lot 3, control	Lot 4, stored cold 4 weeks	Lot 5, stored cold 4 weeks and warm 1 week	Lot 6, stored cold 4 weeks and warm 2 weeks	Lot 7, control	Lot 8, first stored cold 4 weeks then—	
								One half stored warm 4 weeks	One half stored warm 8 weeks
	[a]3	7	2	8	6	6	1	----	----
	3	6	2	7	7	6	1	3	3
	2	5	3	7	7	6	1	6	2
	3	6	3	8	6	5	3	6	2
	2	7	2	8	6	6	2	6	3
	2	7	3	8	6	5	2	6	2
	2	6	2	8	7	6	2	----	----
	2	6	3	7	6	6	3	----	----
	2	6	2	7	7	6	1	----	----
	2	6	2	7	6	6	2	----	----
Average	2.3	6.2	2.4	7.5	6.4	5.8	1.8	5.4	2.4

LINE 10

	2	7	2	8	6	7	1	----	3
	1	7	2	7	6	6	2	----	3
	1	7	2	7	6	5	2	----	3
	1	7	1	7	6	6	1	----	3
	1	7	2	7	7	5	1	----	3
Average	1.2	7	1.8	7.2	6.2	5.8	1.4	----	3

[a] For meaning of humerals see discussion of color scale (p.482). The larger the number, the darker the color.

Though the tubers of each line were genetically alike in so far as they were descended within three years from the same parent tuber, they did not respond in an identical manner to the conditions of storage.

HILL SELECTION AND CHIP COLOR

Some hills were dug as units and kept separate to compare the color of the chips made from tubers in such groups under the different storage conditions. The results are shown in Table 6.

TABLE 6.—*Storage effects on color of chips made from tubers grown in a single hill*

[Each lot is from one hill]

LOT 1, CONTROL

Tuber No.	Weight	Length	Width	Depth	Color
	Grams	*Cm.*	*Cm.*	*Cm.*	
1	51	4.8	4.7	3.6	[a]1
2	56	4.9	5.2	3.9	1
3	81	5.0	5.8	4.8	1
4	139	7.0	7.7	4.9	1
5	143	7.5	6.6	5.0	1
6	173	7.8	7.1	5.2	1
Average					1

LOT 2, STORED COLD 2 WEEKS

1	23	3.6	3.7	3.1	7
2	41	4.6	4.4	3.4	7
3	80	5.0	6.1	4.3	5
4	81	4.4	6.0	4.4	7
5	160	7.8	7.1	4.8	6
6	170	7.4	7.4	5.2	6
Average					6.3

LOT 3, CONTROL

1	24	3.7	4.0	2.7	4
2	59	5.2	5.1	3.6	2
3	68	5.8	5.4	3.9	4
4	78	5.2	5.9	4.2	2
5	123	7.2	6.2	5.0	3
6	212	8.9	7.4	5.4	2
7	209	8.7	7.5	5.5	3
Average					2.9

LOT 4, STORED COLD 4 WEEKS

1	32	4.5	3.8	3.3	8
2	81	5.7	5.6	4.5	7
3	145	7.0	6.8	5.0	7
4	147	6.6	6.9	5.1	7
Average					7.2

LOT 5, STORED WARM 1 WEEK AFTER COLD STORAGE FOR 4 WEEKS

Tuber No.	Weight	Length	Width	Depth	Color
	Grams	*Cm.*	*Cm.*	*Cm.*	
1	63	4.5	5.5	4.8	7
2	204	9.6	6.8	5.1	6
3	223	9.5	7.3	5.3	6
4	237	9.0	7.7	6.0	7
5	249	9.2	8.1	5.7	6
Average					6.4

LOT 6, STORED WARM 2 WEEKS AFTER COLD STORAGE FOR 4 WEEKS

1	129	6.8	6.8	4.5	6
2	198	6.0	7.5	7.5	6
3	298	10.5	8.1	6.3	6
Average					6

LOT 7, CONTROL

1	11	2.2	3.0	2.3	3
2	17	2.7	3.2	2.7	3
3	87	5.9	5.5	4.2	3
4	110	6.4	6.5	4.7	1
5	118	6.5	6.3	4.8	1
6	120	6.6	6.6	4.6	2
7	149	7.2	7.1	4.9	1
Average					2

LOT 8, STORED WARM 4 WEEKS AFTER COLD STORAGE FOR 4 WEEKS

1	34	3.9	4.3	3.3	4
2	39	4.7	4.1	3.2	3
3	62	4.8	5.5	3.8	3
4	50	4.8	5.4	3.4	5
5	80	7.9	6.4	4.1	5
6	90	6.1	5.7	4.6	3
7	120	7.2	6.4	4.3	4
Average					3.9

[a] For meaning of numerals see discussion of color scale (p. 482). The larger the number, the darker the color.

There is considerable variation in response to effects of storage on color of chips made from tubers produced in a single hill, in some cases more than in others. However, averages show the same trend indicated by the averages of the 10-tuber samples used in the other lots.

Records for the individual tubers were taken where the data are summarized as in Tables 2, 3, and 4, but, as these in Table 6 indicate, there is no correlation between size and shape and the color of the chips.

Disease and Chip Color

The foregoing data were secured on potatoes raised in plots in which there was no recognizable disease, or in which all diseased plants were rogued out early in the season, as explained in the discussion of selection of the potatoes. A less complete set of experiments was carried out on samples from hills where there was known disease and from healthy rows growing adjacent to such diseased plants. These data are given in Table 7.

Table 7.—*Effect of storage under different conditions on color of chips made from diseased potato stock, or healthy stock grown near diseased*

Growth conditions as related to disease	Control	Stored cold 2 weeks	Stored warm 2 weeks after cold storage 2 weeks
Spindle tuber (A)[a]	[b]3.1	5.7	2.5
Spindle tuber (H)[a]	1.4	3.3	2.2
Healthy next spindle tuber (A)	3.5	6.9	3.4
Healthy next spindle tuber (H)	2.2	3.5	3.4
Leaf roll (A)	3.1	6.0	3.8
Leaf roll (H)	2.8	4.1	2.2
Healthy next leaf roll (A)	4.6	7.0	4.0
Healthy next leaf roll (H)	2.3	4.9	2.9
Rugose mosaic (A)	4.0	5.5	5.0
Rugose mosaic (H)	2.3	2.1	2.6
Healthy next rugose mosaic (A)	4.3	6.3	3.5
Healthy next rugose mosaic (H)	1.8	3.0	3.1
Mild mosaic (A)	3.3	5.0	3.5
Mild mosaic (H)	1.2	3.4	2.8
Healthy next mild mosaic (A)	4.7	6.4	3.4
Healthy next mild mosaic (H)	1.9	3.4	2.4
Giant hill (H)	2.6	5.7	4.3
Healthy next Giant hill (H)	2.2	5.4	2.6
Healthy (A)	4.0	6.4	3.9

[a] (A)=potatoes grown at Aroostook farm; (H)=potatoes grown at Highmoor farm.
[b] For meaning of numerals see discussion of color scale (p. 482). The larger the number, the darker the color.

In a few cases disease apparently affected the rate of change of frying color. Both samples of rugose-mosaic tubers failed to exhibit much change in the period allowed. One sample from healthy stock planted near stock having this disease showed this effect. These results should be tested further before they are considered especially significant.

Correlation of Chip Color with Sugar Content of Tubers

Five tuber lots of Green Mountain, from which sample chips corresponded to each color in the scale, were analyzed for their sugar content. The results are given in Table 8.

Table 8.—*Sugar content of Green Mountain tubers producing chips of standard colors*

Chip No.[a]	Total sugar in tubers[b] (wet weight)	Chip No.[a]	Total sugar in tubers[b] (wet weight)
	Per cent		*Per cent*
1	0.19	5	0.60
2	.27	6	2.87
3	.36	7	3.14
4	.48	8	3.25

[a] See discussion of color scale for significance of numerals (p. 482). The larger the number, the darker the color.
[b] Sugar analyses made by E. R. Tobey, research chemist, Maine Agricultural Experiment Station.

The higher the sugar content of the potatoes, the browner were the chips made from them. Thus low sugar content appears to be associated with lightness of color, whereas potatoes containing a high sugar content produce dark chips.

The comparatively large increase in sugar content of tubers corresponding in chip color to No. 6 as compared with No. 5 is not surprising when one recalls that chip No. 6 was selected from those made from tubers that had been in cold storage, while the first five chips were made from tubers as they came in from the field.

The correlation of browning during cooking at high temperatures with the sugar content of potatoes is similar to the condition found in baking bread. The amount of residual sugar escaping yeast fermentation depends upon the diastatic activity of the flour. Flours possessing high diastatic activity have high residual sugar and consequently make breads which brown easily during baking (*3*).

TRADE PREFERENCES IN CHIP COLOR

In order to ascertain trade preferences in color of potato chips, nine sets of chips similar to those used in the color scale for rating the experimental products were prepared and sent out to a corresponding number of manufacturers. The eight reports that were returned are summarized in Table 9.

TABLE 9.—*Trade preferences for potato chips according to color*

Manufacturer No.	Colors rated—			Manufacturer No.	Colors rated—		
	Most desirable[a]	Marketable	Unmarketable		Most desirable[a]	Marketable	Unmarketable
1	1	1, 2, 3	4, 5, 6, 7, 8	5	1, 2	1, 2, 3, 4	5, 6, 7, 8
2	1, 2, 3	1, 2, 3, 4	5, 6, 7, 8	6	3	1, 2, 3	4, 5, 6, 7, 8
3	1, 2	1, 2, 3	4, 5, 6, 7, 8	7	2	1, 2, 3	4, 5, 6, 7, 8
4	2, 3	1, 2, 3, 4	5, 6, 7, 8	8	2, 3	2, 3, 4	5, 6, 7, 8

[a] See discussion of color scale for significance of numerals (p. 482). The larger the number, the darker the color.

Potato-chip manufacturers agreed that the trade prefers a light chip, so light that in a northern climate potatoes may be undesirable for this purpose even at digging time. (Compare these data with those in Table 2.)

From Table 8 it also seems that in the potatoes sampled, a sugar content of one-half of 1 per cent or over is associated with a chip that is unmarketable on account of its color.

DISCUSSION

It is well known that potatoes freeze at about 29° F. (*11*), and that the formation of ice crystals causes definite injury to cooking quality, but it is not so widely appreciated that storage at temperatures well above this will cause internal chemical changes which affect culinary value. In his study of the carbohydrate transformations in potatoes Appleman (*1*) found that sugar accumulates at the expense of starch when the temperature falls to 38°, and that the rate of accumulation is most rapid between 32° and 29°. In four to six weeks a maximum

of 3 to 4 per cent sugar is reached. Hopkins (*4*) found that at 40° the sugar content was fairly constant.

Stuart et al. (*8*) reported that the temperature of a potato-storage house on Aroostook farm varied between 34° and 45° F. during a winter, and so it is evident that storage houses in northern regions give conditions in which sugar accumulation takes place. Furthermore, such storage temperatures are considered desirable to prolong dormancy and to delay sprouting.

Applemen (*1*) also found that potatoes which have accumulated sugar at low temperatures will lose four-fifths of this by reconversion to starch and the remaining one-fifth through respiration in a room at 70° to 75° F. in a week's time. At lower temperatures, 45° to 50°, a much longer exposure is necessary. This phenomenon probably explains the lighter color secured when tubers from lots from which dark chips had been made were held at room temperatures. In this study the constancy of the controls indicates that the loss of sugar at the temperatures of the storage cellar (40° to 55°) was much smaller than the conclusions of Appleman (*1*) and Hopkins (*4*) would lead one to expect.

Appleman and Miller (*2*) in analyses of potatoes dug at different stages of development found that the percentage (wet weight) of reducing sugar plus sucrose was 0.991 in those dug on June 17, whereas those dug on August 27 contained only 0.255 per cent of these sugars. These were Irish Cobbler potatoes grown in Maryland, and the vines were brown and dry on the latter digging date.

The writer's experiments on making chips from potatoes dug at various times showed that the chips from those dug at the earlier dates were darker. (Table 4.) Again, this is a difference associated with relative sugar content. Differences in those dug earlier would probably have been more outstanding but for the period of holding before frying, as Appleman and Miller also say that the percentage of sucrose in their lots evened up by decrease during storage.

The factor of immaturity probably contributes to the difficulty in securing satisfactory chips from northern-grown potatoes even immediately after digging and before winter storage has produced its effects. Frequently these potatoes are dug while the vines are yet green. The difference in maturity may explain the difference in color of the chips made from Green Mountain potatoes produced at Highmoor farm and those grown on Aroostook farm. (Tables 2 and 3.) The difference in soil temperature may also be a factor.

Since the carmelization of sugar is more rapid and complete at high temperatures than at low, the practicability of advocating lower frying temperatures was considered. Woodruff and Blunt (*10*) found that the overbrowning produced when potatoes were fried at 190° to 210° C. (374°–410° F.) diminished when they were fried at 170° to 190° (338°–374°). In the range of temperatures used, fat absorption did not vary with temperature.

In two tests on Green Mountain potatoes that had been in cold storage the author found that there was less browning when chips were fried at temperatures below 320° F. Fat absorption did not increase with lower temperatures until the temperature of the fat was below 250°. However, the sweetish flavor of the larger amount of sugar was evident and was objectionable.

SUMMARY

By employing the standard Munsell color disks it was found possible to measure the color of potato chips as a scale for comparison.

When potatoes of three varieties were held in commercial cold storage two weeks or more all produced chips very much darker than those made from tubers of the same lot cooked at the beginning of the experiment or held in storage at 40° to 55° F. If the potatoes which had been in cold storage were held at room temperature, the frying color became lighter. This change in color is correlated with change in sugar content under these different conditions of storage.

Potatoes dug at an early date so that they were somewhat immature gave chips darker than those from mature tubers. Size of tuber was not significantly related to chip color in the more mature lots.

Individual tubers from the same tuber line or from the same hill did not respond in an identical manner to those differences in storage temperatures. Averages were similar to those in other lots receiving comparable treatment.

Tubers from plants showing signs of the diseases spindle tuber, leaf roll, rugose mosaic, mild mosaic, and giant hill responded to cold storage like the healthy stocks already discussed except those having rugose mosaic. These did not show much change.

A survey of trade preferences showed that very light chips are desired. Northern-grown potatoes may be unsuitable for this purpose when they are brought in from the field. (See lot 1, Tables 2 and 3.)

LITERATURE CITED

(1) Appleman, C. O.
1912. changes in potatoes during storage. Md. Agr. Expt. Sta. Bul. 167, p. [327]–334.

(2) ——— and Miller, E. V.
1926. a chemical and physiological study of maturity in potatoes. Jour. Agr. Research 33: 569–577, illus.

(3) Blish, M. J., Sandstedt, R. M., and Platenius, H.
1929. correlation between diastatic power of flour and crust color in the test loaf, and its significance. Cereal Chem. 6: 121–127.

(4) Hopkins, E. F.
1924. relation of low temperatures to respiration and carbohydrate changes in potato tubers. Bot. Gaz. 78: [311]–325, illus.

(5) Nickerson, D.
1929. a method for determining the color of agricultural products. U. S. Dept. Agr. Tech. Bul. 154, 32 p., illus.

(6) Schultz, E. S.
1924. why potatoes run out. U. S. Dept. Agr. Farmers' Bul. 1436, 21 p., illus.

(7) ——— and Folsom, D.
1923. transmission, variation, and control of certain degeneration diseases of Irish potatoes. Jour. Agr. Research 25: 43–117, illus.

(8) Stuart, W., Lombard, P. M., and Peacock, W. M.
1929. comparative influence of different storage temperatures on weight losses and vitality of seed potatoes. U. S. Dept. Agr. Tech. Bul. 117, 18 p., illus.

(9) Vosbury, M. C.
1922. methods of manufacturing potato chips. U. S. Dept. Agr. Bul. 1055, 20 p., illus.

(10) Woodruff, S., and Blunt, K.
1919. changes in fat absorbed by dried foods. Jour. Home Econ. 11: 440–452, illus.

(11) Wright, R. C., and Diehl, H. C.
1927. freezing injury to potatoes. U. S. Dept. Agr. Tech. Bul. 27, 24 p., illus.

THE CHEMICAL COMPOSITION OF GRASS FROM PLOTS FERTILIZED AND GRAZED INTENSIVELY IN 1929 [1]

By J. G. ARCHIBALD [2]

Assistant Research Professor of Chemistry, Massachusetts Agricultural Experiment Station

INTRODUCTION

The findings reported in this paper were obtained during the season of 1929 in the continuation of a project, the first results of which were reported about a year ago.[3]

For an outline of experimental methods connected with the project and detailed discussion of the results obtained in 1928 the reader is referred to the earlier paper.

Briefly, the conclusions reached were:

Application of fertilizer increased the quantity of dry matter produced and of all its constituents and improved the quality of the herbage.

Seasonal fluctuations in composition of the herbage were not smoothed out to any great degree by application of fertilizer.

Grass kept in the vegetative stage by grazing may vary considerably in composition as the growing season advances on account of the effect of such seasonal factors as rainfall, temperature, sunshine, and length of day.

TABLE 1.—*Kind of fertilizer used, rate, and date of application*

Plot No.	Kind of fertilizer	Pounds per acre applied	Dates of application (1929)
1, 2, 3, 5, 7, 8, and 9	Nitrophoska II [a]	250	Apr. 20–May 2.
	Calurea [b]	[c] 44	At intervals throughout the season varying from 26 days to 56 days, commencing May 28, ending Aug. 29.
	Calurea	44	
	Calurea	44	
4	Check	(d)	
6	Acid phosphate	[e] 258	May 2.
	Muriate of potash	[e] 100	Do.

[a] Nitrophoska II contains 16.5 per cent N, 16.5 per cent P_2O_5 and 20 per cent K_2O.

[b] Calurea contains 34 per cent N and 13 per cent lime (CaO).

[c] Plots 8 and 9 received a double application of Calurea (88 lbs. each) on Aug. 12 and 10, respectively, and none thereafter, thus making the total application to these two plots 132 pounds, the same quantity of Calurea as was applied to plots 1, 2, 3, 5, and 7

[d] No fertilizer.

[e] These amounts of acid phosphate and muriate of potash supplied the same amounts of P_2O_5 and K_2O as did the Nitrophoska II.

[1] Received for publication May 9, 1930; issued October, 1930. Published with the permission of the director of the Massachusetts Agricultural Experiment Station.

[2] Grateful acknowledgment is here made of the cooperation of the Department of Animal Husbandry of Massachusetts Agricultural College and the feed control laboratory of this station, more particularly of the services of R. C. Foley, who took all samples, and of J. W. Kuzmeski and J. B. Zielinski, who made the determinations of crude fiber and ether extract under the direction of P. H. Smith, chief chemist of the feed control laboratory.

[3] ARCHIBALD, J. G., and NELSON, P. R. THE CHEMICAL COMPOSITION OF GRASS FROM PLOTS FERTILIZED AND GRAZED INTENSIVELY. Jour. Amer. Soc. Agron. 21: 686–699, illus. 1929.

EXPERIMENTAL PROCEDURE

The only important variations in the procedure in 1929 as compared with that in 1928, were the omission of nitrogen from the fertilizer application for Plot 6 and a 25 per cent reduction in the amounts of phosphoric acid and potash applied to the other fertilized plots. Also less nitrogen was applied in the spring and more during the summer. The detailed fertilizer schedule for 1929 appears in Table 1.

The system of grazing and of taking grass samples was identical with that in use in 1928.

DISCUSSION OF RESULTS

Results will be viewed from two angles: The effect of the fertilizer; and seasonal variations.

As in 1928, results from plots 7, 8, and 9 have been kept separate because these plots were not grazed during the early part of the season, being reserved for supplementary grazing acreage later in the summer. The grass on them was cut for hay in June. The cautionary note sounded in the earlier publication [4] regarding interpretation of results is equally applicable here and need not be repeated. Table 2 shows the effect of the fertilizer treatment.

TABLE 2.—*Effect of fertilizers on the chemical composition (on a dry basis) of pasture grass grown on experimental plots in 1929*

[As in 1928 the values expressed in pounds per acre are averages of the weights of the crop at the several times of sampling. Obviously total weights would not be comparable because of the varying number of samples in the different groups]

Plot and fertilizer used	Dry matter		Nitrogen		Crude fiber		Ether extract		Calcium		Phosphorus	
Plots 1, 2, 3, and 5	*Per cent*	*Lbs. per acre*	*Per cent*	*Lbs. per acre*	*Per cent*	*Lbs. per acre*	*Per cent*	*Lbs. per acre*	*Per cent*	*Lbs. per acre*	*Per cent*	*Lbs. per acre*
Average of 28 samples (Nitrophoska, Calurea.)	28.0	1,102	3.0	33.2	23.7	261	3.2	35.1	0.64	7.0	0.31	3.4
Plots 7, 8, and 9												
Average of 14 samples (Same fertilizer treatment as above, but not grazed until July.)	34.1	1,126	2.4	27.2	25.4	287	3.0	33.6	.67	7.6	.22	2.4
Plot 6 (no nitrogen)												
Average of 5 samples (Acid phosphate, muriate of potash.)	33.4	951	2.5	23.8	23.9	227	3.4	32.3	.65	6.2	.26	2.4
Plot 4 (check)												
Average of 5 samples (Unfertilized.)	39.8	971	1.9	18.4	26.7	260	2.9	27.9	.58	5.6	.27	2.6

Comparison of the figures shows that—

The grass from the check plot contained about 42 per cent more dry matter than did that from plots 1, 2, 3, and 5, but the average yield of dry matter on the dates when the samples were taken was about 13 per cent greater on the latter. Withholding nitrogen on plot 6 resulted in a dry matter percentage of 33 per cent as compared

[4] ARCHIBALD, J. G., and NELSON, P. R. Op. cit., p. 689.

with 28 per cent in plots 1, 2, 3, and 5, and a dry matter production of 151 pounds less per acre, or, in other words, less succulent grass and less of it, even on a dry-matter basis. The considerably higher dry-matter content of the grass from all plots in 1929 as contrasted with 1928 can be attributed to a season as abnormally dry as that of 1928 was abnormally wet.

There was 58 per cent more nitrogen in the dry matter from plots 1, 2, 3, and 5 than in that from the check plot, and about 80 per cent more nitrogen was recovered per acre. This is an even greater response to the fertilizer than was obtained in 1928 and seems to point to one of two things or perhaps to both: (1) A residual nitrogen effect carrying over from the previous season, probably the result of a better developed sod on the fertilized plots, due to the fertilizer treatment in 1928; and (2) nitrogen applications may be more effective in a dry season than in a wet one.

That the first mentioned of these possibilities, viz, the residual nitrogen effect, must be at least in part responsible for the difference, seems evident from a comparison of the results from plot 6, which did not have nitrogen in 1929 but did have it in 1928, with the results from the completely fertilized plots and the check plot. A nitrogen content about midway between the two extremes was found in the grass from this plot, and an acre recovery of nitrogen about 29 per cent greater than the recovery on the check plot, albeit much below that on plots 1, 2, 3, and 5.

Production of crude fiber was about the same on plots 1, 2, 3, and 5 as on the check plot, but the percentage in the dry matter was somewhat less (23.7 as against 26.7), indicating that the grass had a higher nutritive value where the fertilizer was applied. Omission of nitrogen on plot 6 caused very little increase in fiber percentage—only 0.2 per cent. It should not be concluded, however, that nitrogen is unnecessary for production of high quality grass, for, as shown in the preceding paragraph, there was a very evident carry-over of nitrogen from the previous season on this plot.

While the crude fiber content of the dry matter of the grass from the fertilized plots (1, 2, 3, and 5) was about the same in 1929 as in 1928 (23.7 per cent and 23.5 per cent), there was a marked increase the second season for the check plot, 26.7 per cent as compared with 25 per cent. This is probably chargeable to the very dry season in 1929 and indicates that the check plot did not meet the severe test of the drouth so satisfactorily as did the fertilized plots. The very much higher dry-matter content on this plot in 1929 than in 1928 leads to the same conclusion.

The ether extract in the dry matter was about 10 per cent higher on plots 1, 2, 3, and 5 than on the check, and production per acre was about 26 per cent higher. Relatively these results are similar to those obtained in 1928, but despite the dry season the actual amount of ether extract produced was somewhat higher for all plots than in 1928. Regarding the intermediate position of plot 6 a deduction can be made similar to that already mentioned for nitrogen and crude fiber. Respecting the percentage content of ether extract, plot 6 was higher than the completely fertilized plots. It seems probable that this difference is relative only, because of the formation of less nitrogenous

substance where the nitrogen was withheld and a corresponding relative increase in nonnitrogenous substances.

The content of calcium was also about 10 per cent higher on the fertilized plots than on the check (a reversal of the findings in 1928), and recovery per acre was 25 per cent higher. The percentage and recovery of calcium were higher on all plots, the check included, than in 1928.

The phosphorus content was about 15 per cent higher on plots 1, 2, 3, and 5 than on the check, and recovery of phosphorus was 31 per cent greater. All plots showed a decrease in content but an increase in recovery of phosphorus as compared with 1928. The decrease in phosphorus content is possibly a reflection of the smaller amount of phosphate fertilizer applied in 1929 but is more likely due to the severe drought of July and August. Orr[5] in a recent review of the subject cites several investigators whose work points to a lowered phosphorus content in pasture herbage during serious droughts.

With reference to plots 7, 8, and 9 the principal reason for the variation between these and plots 1, 2, 3, and 5 is a seasonal one, samples not having been taken from them until July. The relatively high calcium and low phosphorus which characterized these plots in 1928 persisted in 1929. As suggested in the earlier paper the high calcium may be due to the liberal application of calcium nitrate which these plots received in 1928. The low phosphorus may perhaps be accounted for by the fact that these plots have been brought under cultivation comparatively recently and as a consequence may not have accumulated a phosphorus reserve comparable with that on the first six plots, which had been fertilized quite liberally over a considerable period of years previous to the commencement of the grazing experiment.

To summarize briefly, the dry matter of the grass from the fertilized plots (1, 2, 3, and 5) was higher in total nitrogen, ether extract, calcium, and phosphorus, and lower in crude fiber than the dry matter of the grass from the unfertilized plot. Acre production of all constituents determined, crude fiber excepted, was markedly higher on the fertilized plots than on the check. For the most part the difference between fertilized plots and check was greater than in 1928. The effect of withholding nitrogen from plot 6 in 1929, as would be expected, was most apparent in the nitrogen results from that plot

SEASONAL VARIATIONS

A summary of the seasonal variations appears in Table 3. The rainfall and temperature records at Amherst for the growing season of 1929 are shown in Figure 1 and have been given careful consideration in accounting for variations in composition. Variations in the several constituents are considered in detail below and are further illustrated in Figures 2–7.

[5] ORR, J. B. MINERALS IN PASTURES AND THEIR RELATION TO ANIMAL NUTRITION. p. 41–44. London. 1929.

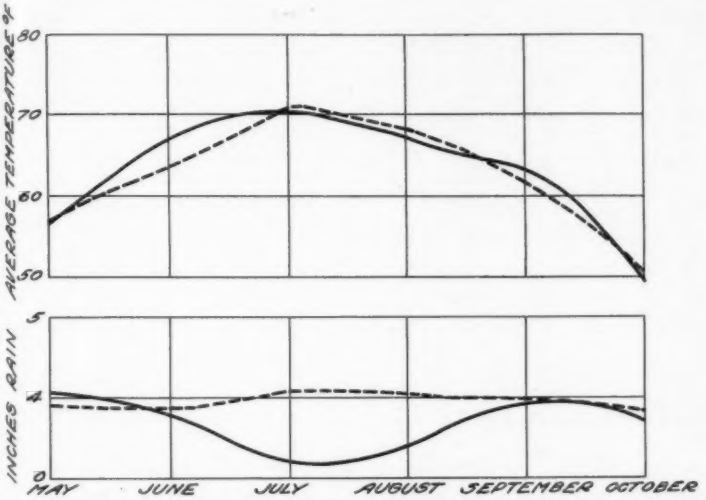

FIGURE 1.—Rainfall and temperature records at Amherst during the growing season of 1929. Dotted lines represent normals for Amherst; these vary slightly from those given for last year since a new 40-year normal has been established, whereas the 1928 weather graphs were based upon the 25-year normal formerly used by this station

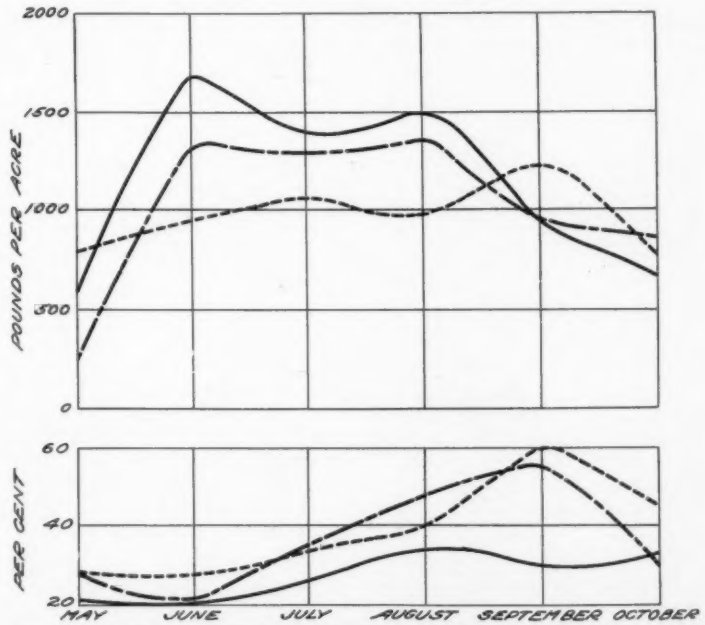

FIGURE 2.—Variations in average percentage content and production in pounds per acre of dry matter in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot

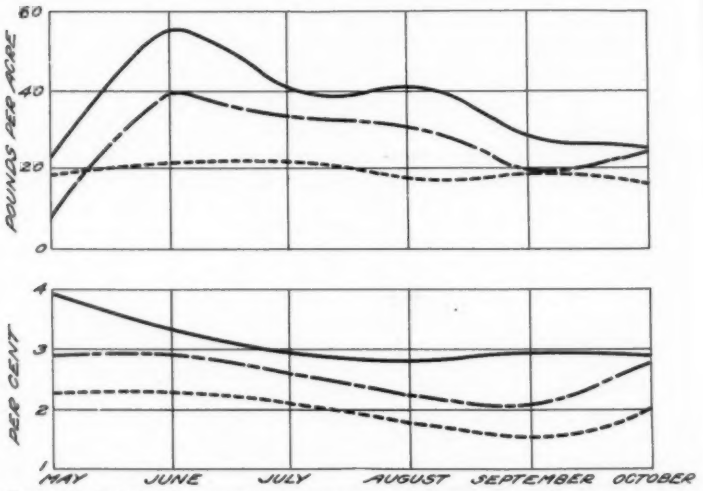

FIGURE 3.—Variations in average percentage content and recovery in pounds per acre, on a dry basis, of nitrogen in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot

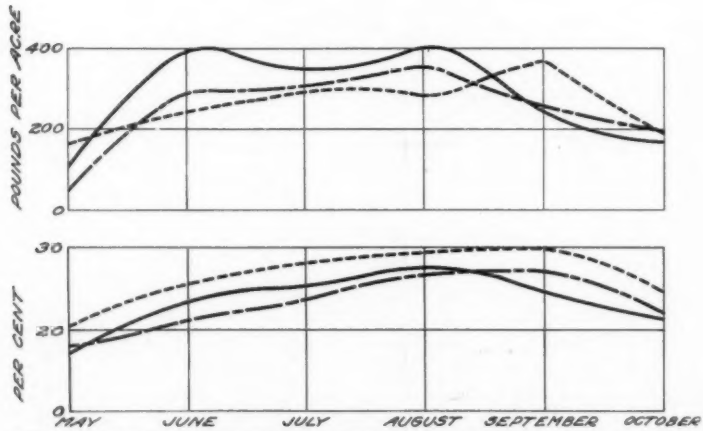

FIGURE 4.—Variations in average percentage content and recovery in pounds per acre, on a dry basis, of crude fiber in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot

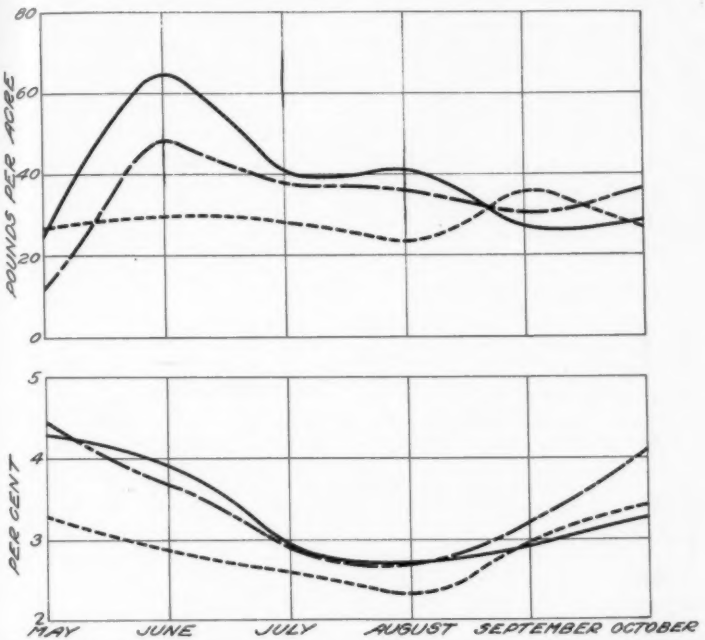

FIGURE 5.—Variations in average percentage content and recovery in pounds per acre, on a dry basis, of ether extract in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot

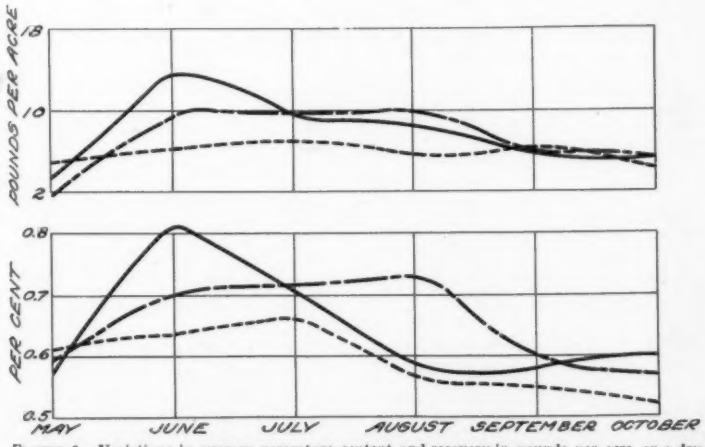

FIGURE 6.—Variations in average percentage content and recovery in pounds per acre, on a dry basis, of calcium in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot

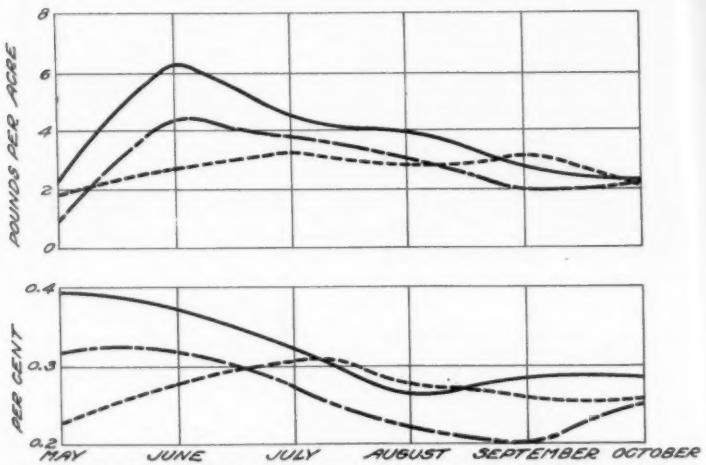

FIGURE 7.—Variations in average percentage content and recovery in pounds per acre, on a dry basis, of phosphorus in grass as affected by fertilization of plots. The solid line represents completely fertilized plots, the dot-dash line the no-nitrogen plot, and the dotted line the check plot

TABLE 3.—*Seasonal variations in chemical composition (on a dry basis) of pasture grass grown on plots fertilized with and without nitrogen and on check plots, in 1929*

PLOTS 1, 2, 3, AND 5

Month	Dry matter		Nitrogen		Crude fiber		Ether extract		Calcium		Phosphorus	
	Per cent	*Lbs. per acre*	*Per cent*	*Lbs. per acre*	*Per cent*	*Lbs. Per acre*	*Per cent*	*Lbs. per acre*	*Per cent*	*Lbs. per acre*	*Per cent*	*Lbs. per acre*
May	22.0	583	3.9	22.7	17.4	102	4.3	24.8	0.57	3.3	0.39	2.3
June	23.3	1,682	3.3	55.0	23.1	388	3.9	64.7	.81	13.7	.37	6.2
July	27.4	1,385	2.9	39.9	25.1	348	2.9	39.9	.70	9.6	.32	4.4
August	34.6	1,491	2.8	41.1	27.3	407	2.7	40.8	.58	8.6	.26	3.9
September	29.6	933	2.9	27.3	23.9	223	2.9	26.6	.56	5.2	.28	2.6
October	33.6	676	2.9	24.3	20.4	170	3.3	27.7	.60	5.0	.28	2.3
PLOTS 7, 8, 9[a]												
July	28.5	1,315	2.4	31.2	25.0	343	2.9	37.6	0.86	11.3	0.23	3.0
August	41.9	1,219	2.3	24.3	27.6	337	2.8	33.7	.60	7.3	.21	2.5
September	32.7	1,002	2.4	24.3	21.8	248	3.2	32.4	.55	5.5	.21	2.1
October	41.6	1,023	2.4	24.5	22.8	233	3.2	32.8	.63	6.4	.21	2.1
PLOT 6 (NO NITROGEN)[b]												
May	27.8	268	2.9	7.8	18.1	48	4.4	11.7	0.59	1.6	0.32	0.9
June	22.0	1,323	2.9	38.6	21.7	287	3.7	48.4	.70	9.2	.32	4.3
July[c]												
August	47.6	1,352	2.2	30.2	25.4	357	2.7	35.8	.73	9.8	.22	3.0
September	55.3	940	2.0	18.7	27.0	254	3.2	29.7	.59	5.5	.19	1.8
October	32.0	871	2.7	23.6	21.9	191	4.1	35.9	.57	5.0	.25	2.2
PLOT 4 (CHECK)[b]												
May	28.2	798	2.3	18.2	23.3	162	3.3	26.5	0.61	4.9	0.23	1.8
June[b]												
July	34.1	1,062	2.1	21.9	27.8	295	2.6	27.9	.66	7.0	.31	3.2
August	39.7	991	1.8	17.3	28.9	286	2.3	22.9	.56	5.5	.28	2.8
September	60.1	1,228	1.5	18.8	29.8	366	2.9	35.7	.55	6.7	.26	3.1
October	45.6	776	2.0	15.7	24.3	189	3.4	26.6	.52	4.1	.26	2.0

[a] As plots 7, 8, and 9 grew a crop of hay during the early part of the season, no samples were taken from them until July and the results from them are not included in the discussion of seasonal variations.

[b] The monthly values for the check plot and the no-nitrogen plot can not be considered as reliable as those for the completely fertilized plots, for they represent analyses of only one sample, whereas the others are averages of from three to five samples.

[c] No sample taken; plot not grazed that month because of insufficient growth of grass.

DRY MATTER

The most striking feature of the fluctuations in dry matter was the remarkable rise in percentage content as the season advanced, more especially in the check and no-nitrogen plots. This of course was a direct and immediate result of the very dry season. The effect of the September rains in lowering the percentage is also worthy of note. Along with the consistently lower dry-matter content of the grass on the fertilized plots should be noted the consistently higher production of dry matter on these plots in pounds per acre except at the end of the season. With the one exception of the check-plot value for June, production of dry matter was consistently higher on all plots than in 1928 and for the most part markedly so.

NITROGEN

Fluctuations in percentage content of nitrogen showed the same general trend as in 1928, although they were not so marked. The completely fertilized plots were consistently higher than the no-nitrogen plot, and it in turn was higher than the check plot respecting both nitrogen content and recovery of nitrogen all through the season. The effect of residual nitrogen on the no-nitrogen plot referred to on p. 493 is even more strikingly brought out by a study of the seasonal fluctuations. Of interest is the increase in nitrogen content in October on the check and no-nitrogen plots. This is attributed to the rains and warm weather of early September.

Fluctuations in nitrogen recovery on the fertilized plots were more marked than in 1928 although of a similar trend. The reverse was true of the check plot.

CRUDE FIBER

As the season advanced there was a quite uniform increase in the percentage content of crude fiber on all plots until August and on the check and no-nitrogen plots until September. In 1928 the peak was reached in July and August, respectively, or one month earlier. The difference is probably due to the marked difference in the two seasons.

This marked increase in fiber content as the season progresses was commented upon rather fully in the earlier publication and was the chief basis for a conclusion drawn at that time to the effect that "although grass may be kept short by grazing or other means, it does not follow that it will have a composition and nutritive value throughout the season similar to that it had at the start."

This viewpoint has been questioned by Ellenberger et al.[6] on the ground that the grass may have been more mature later in the season. This possibility was recognized in our former publication where it was stated that "part of the variation may have been due to slight differences in stage of maturity of the samples." It must be reiterated here, however, that all samples were very immature when taken, being still in the early vegetative stage, and it is difficult to believe that a variation from 17 per cent to 27 per cent of crude fiber could be more than partly accounted for on the basis of stage of maturity. The conclusion drawn in the earlier paper that the process of fiber building in the plant "is, in part at least, seasonal and to that extent inde-

[6] ELLENBERGER, H. B., NEWLANDER, J. A., and JONES, C. H. YIELD AND COMPOSITION OF PASTURE GRASS. Vt. Agr. Expt. Sta. Bul. 295, 68 p., illus. 1929.

pendent of the stage of maturity of the plant," seems to be further confirmed by the results in 1929.

Acre production of crude fiber showed very similar seasonal fluctuations in all plots irrespective of fertilizer treatment.

ETHER EXTRACT

On all plots the seasonal fluctuations in content of ether extract parallel very closely the amount of rainfall, showing how dependent is the synthesis of this particular constituent on the moisture supply. The same was true in general last year. The ether extract of plant materials always includes whatever pigments may be present, the most important one in grass being chlorophyll. When one reflects on how grass "greens up" after a rain, indicating rapid elaboration of chlorophyll, the relationship between content of ether extract and amount of rainfall is seen to be a rational one.

The fertilizer treatment maintained acre production of this constituent at a much higher level during the first part of the season, but seasonal variations were accentuated rather than diminished by it.

CALCIUM

The seasonal variations in calcium were entirely different from the results of 1928, again due presumably to great seasonal contrasts in moisture supply. On all plots a relatively low value was found in May, which on the fertilized plots suddenly increased to a much higher value in June, was slower in reaching a maximum on the check and no-nitrogen plots, and dropped rapidly from the maximum in all cases to values at the end of the season as low or lower than those found at the start. The same was substantially true for the acre recovery of this element.

PHOSPHORUS

Unlike calcium, phosphorus showed much the same seasonal trends as it showed in 1928. Fluctuations in the values for the fertilized plots showed an even more striking resemblance to those for nitrogen than they showed in 1928, and with respect to the check plot the same apparent inconsistency is evident as was commented upon in the earlier publication, viz, a low value at the beginning of the season, rising to a maximum in midsummer. This situation is hard to account for; last season any attempt at explanation was carefully avoided as it was considered unsafe to draw conclusions until further results had been secured. Since a similar result has been obtained the second year it may be safe to put forward a tentative explanation to the effect that the liberal supply of plant food on the fertilized plots had its maximum effect in the earlier part of the growing season, while the rate of metabolic activity was somewhat slower on the depleted check plot.

SUMMARY

Results of analyses of 52 samples of grass from nine 8-acre pasture plots that were fertilized and grazed intensively, are reported, discussed, and compared with results which were secured in the previous season (1928), and which have been published elsewhere. The fertilizer treatment was the same as in 1928 except that nitrogen was withheld from one plot that had a complete fertilizer that year, and

the amounts of phosphoric acid and potash were reduced 25 per cent on all plots. In other respects the experimental procedure was the same.

The fertilizer treatment decreased considerably the dry-matter content of the grass and the crude fiber content of the dry matter. All other constituents of the dry matter that were determined were increased, the nitrogen showing the most significant rise. Acre production or recovery of all constituents was increased, nitrogen being nearly double that recovered on the check plot. The effect of withholding nitrogen from one plot was very marked.

The conclusions reached in the earlier publication regarding (1) increase in nutritive value due to fertilization, (2) absence of any appreciable effect of the treatment in leveling seasonal fluctuations, and (3) changes in chemical composition of the grass as the season advanced, are substantiated and strengthened by the results of 1929.

A careful study of all results for both seasons, and correlation of these with the weather records leads to the further conclusion that of all the factors that influence the chemical composition of grass, producing as they do marked seasonal fluctuations, the amount of rainfall is the most important.

THE USE OF PRESERVATIVES TO PREVENT LOSS OF NITROGEN FROM COW EXCRETA DURING THE DAY OF COLLECTION [1]

By ROWLAND B. FRENCH

Associate in Animal Nutrition, Institute of Animal Nutrition, Pennsylvania State College

INTRODUCTION

This study was undertaken at the request of Director E. B. Forbes of this institute, in the course of preparations for a series of mineral and nitrogen balances with cows, and had for its object the determination of a method of prevention of the loss of nitrogen from combined urine and feces. These excreta, as voided, were to be guided by a rubber apron and galvanized-iron hopper into an open galvanized-iron pan in the gutter behind the cow; and the conditions to be met by the treatment were that it be effective during an interval of 24 hours; and that the preservative be cheap and free from objectionable odor, and of such nature as to be easily handled, and as not to contain nitrogen or any of the several inorganic elements to be studied.

METHODS

In the preliminary tests samples of fresh feces and urine were mixed in equal proportions and allowed to stand in open vessels at about 70° F. for 18 hours. Kjeldahl determinations then revealed losses which ranged from 6 to 30 per cent of the total nitrogen present. This large variation in the loss of nitrogen from the several samples was caused by differences in surface exposed—the greater the surface in relation to the quantity of excreta the greater the loss of nitrogen. Nitrogen determinations in further experiments were easily performed with the aid of a Folin ammonia apparatus, as described later.

The sample of excreta was put into an aeration flask, and ammonia-free air was drawn over the surface of the sample and through standard sulphuric acid. After titrating the unused sulphuric acid with standard alkali the ammonia lost by the sample was computed. Fifty grams of fecse and 50 c. c. of urine were found to make a convenient-sized sample.

The feces from milk-producing cows was sufficiently plastic to allow a sort of pipetting, by the use of a 12-inch piece of ½-inch glass tubing fitted internally with a movable section of rubber stopper. One end of the tube was closed with this rubber disk and thrust into a mass of feces. Then suction was applied at the open end of the tube sufficient to cause the feces to enter and to rise to a fixed mark. The tube was then introduced into the aerating flask, and the feces were expelled by pushing down the rubber disk with a glass rod. Thus the feces were deposited in the bottom of the flask without smearing them on the neck. To this portion of feces was added 50

[1] Received for publication May 23, 1930; issued October, 1930.

c. c. of urine. A battery of flasks thus prepared was used for a series of preservative comparisons. Since 100 gm. samples were used, and the feces and urine mixtures contained about 1 per cent of nitrogen, the decigrams of nitrogen lost were expressed as per cent of total nitrogen lost—a procedure which is only approximately correct, but which was found useful for comparative purposes.

RESULTS

In Table 1 are given the results of several experiments with different preservatives as listed. The preservative was added in each case in the strength of a normal solution. Then the feces, urine, and preservative were well shaken together.

The temperature of the laboratory varied from 70° to 90° F. at the time of the several experiments.

At the lower concentrations copper sulphate, formaldehyde, and zinc sulphate were most efficient in preventing loss of nitrogen. At the higher concentrations all of the chemicals used showed some preservative action. Copper sulphate was very effective but was discarded because of its reaction with the zinc coating of the collection pans. The tests with formaldehyde and zinc sulphate showed that these preservatives also were effective, and that in sufficient concentration they reduced the nitrogen loss to negligible quantities.

TABLE 1.—*Influence of different preservatives in preventing loss of nitrogen from 100 gm. samples of feces and urine mixtures*

Preservative used	Loss of N, expressed as percentage of total N present, when the indicated quantity of preservative, expressed in cubic centimeters of normal solution per 100 gms. of feces and urine, was used				
	2	4	8	12	15
Copper suphate	0.15				
Boric acid	[a] 3.19				0.31
Ferrous sulphate	[a] 3.19				.08
Formaldehyde	.84	0.18	0.05	0.01	.08
Phenol	[a] 3.19				1.08
Salicylic acid	2.29				.86
Toluene	2.43				1.42
Zinc sulphate	.72	.21	.03	.01	.01

[a] Standard acid completely neutralized, and some ammonia lost.

Check tests without any preservative showed losses of 3.19+ per cent, 5.99 per cent, and 2.63 per cent, in association with the 2 c. c., the 12 c. c., and the 15 c. c. series, respectively.

The manner in which the feces and urine were mixed had a marked effect on the loss of nitrogen as shown in Table 2. The term "not mixed" as used in this table signifies that the urine and preservative were placed in the Kjeldahl flask and that the feces were added thereto without shaking. It is obvious that the thorough mixing of the feces and urine by shaking increases enormously the speed of the action by which the nitrogen is lost, unless prevented by the preservative. Also, in these experiments the loss of nitrogen from the feces and urine, placed together but not mixed, either with or without preservative, was very small.

TABLE 2.—*Loss of nitrogen from cow excreta, expressed as percentage of total nitrogen present, as affected by mixing the urine and feces, and by treatment with 2 c. c. of normal solution of different preservatives per 100 gm. of feces and urine*

Treatment of feces and urine mixtures	Copper sulphate	Boric acid	Ferrous sulphate	Formaldehyde	Phenol	Salicylic acid	Toluene	Zinc sulphate	No preservative (check)
Well mixed	0.15	3.19+	3.19+	0.84	3.19+	2.29	2.43	0.72	3.19+
Not mixed	.35	.72	.12	.10	.55	.46	.19		0.24

In Table 3 are given the results of several experiments in which large quantities of feces and urine were placed in the excreta collection pans. These results serve as a check on the previous smaller-scale experiments. Copper sulphate, zinc sulphate, and formaldehyde gave complete protection against loss of nitrogen when added to a homogeneous mixture of feces and urine. When these excreta were not stirred together, copper sulphate, the most efficient of the preservatives used, showed little, if any, effect in retarding the small loss of nitrogen which occurred. The copper plating of the galvanized-iron collection pans, however, suggested that the pans might not stand up under this treatment.

TABLE 3.—*Loss of nitrogen from large quantities of cow excreta, expressed as percentage of total nitrogen present, as affected by mixing the urine and feces, and by treatment with 2 c. c. of normal solution of different preservatives per 100 gm. of feces and urine*

Treatment of feces and urine mixtures	Copper sulphate	Zinc sulphate	Formaldehyde	No preservative (check)
Well mixed	None.	None.	None.	6 to 30
Not mixed	1.47			[a] 1.0 to 2.6

[a] Temperature variation from 70° to 90° F.

With the elimination of copper sulphate from further consideration, for practical reasons, it was decided to use formalin as a preventive of nitrogen loss. In Table 4 is given a summary of the experiments in which nitrogen was determined with the Folin apparatus and formaldehyde was used as a preservative. The amount of preservative used is given as cubic centimeters of normal solution, per cent as formaldehyde, and per cent as formalin, in the mixtures of excreta, together with the loss of nitrogen from the mixed excreta with and without the preservative.

TABLE 4.—*Results of experiments with formaldehyde as a preservative in preventing loss of nitrogen from feces and urine mixtures*

Quantity of formaldehyde used per 100 gm. of feces and urine mixture			Nitrogen loss expressed as percentage of total nitrogen present [a]	
Normal solution used	Formaldehyde	Formalin (37.5 per cent)	With preservative	Without preservative
C. c.	*Per cent*	*Per cent*	*Per cent*	*Per cent*
2	0.06	0.16	0.84	3.2+
4	.12	.32	.18	6.0
8	.24	.64	.05	6.0
12	.36	.96	.01	6.0
15	.45	1.20	.08	2.6

[a] Nitrogen determinations with Folin apparatus.

SUMMARY

Cow feces and urine, placed together, but unmixed, showed during an 18-hour period losses of from 1 to 2.6 per cent of the total nitrogen present. When these excreta were thoroughly mixed the losses varied from 6 to 30 per cent of the nitrogen.

Copper sulphate, zinc sulphate, and formaldehyde, when added in small concentrations to mixtures of feces and urine, were highly effective in preventing losses of nitrogen as ammonia.

( )

XENOS